PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF ANTI-ULCER ACTIVITY OF FICUS ARNOTTIANA MIQ. FRUIT

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Submitted by

Reg. No.261225706

Under the Guidance of

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DECLARATION

I hereby declare that the dissertation work entitled "***PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF ANTI-ULCER ACTIVITY OF FICUS ARNOTTINA*** **MIQ.** is based on the original work carried out by me under the guidance and supervision of Mr. G.SEKAR M.Pharm., for submission to The Tamilnadu Dr. M.G.R. Medical University, Chennai in the partial fulfillment for the degree of MASTER OF PHARMACY in Pharmacology. This work is original and has not been submitted in part or full for the award of any other degree or diploma of any other university. The information furnished in this dissertation is genuine to the best of my knowledge and belief.

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Introduction

Herbal Medicine sometimes referred to as Herbalism or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. An herb is “a plant or plant part valued for its medicinal, aromatic or savory qualities”. Herb plants produce and contain a variety of chemical substances that act upon the body. **(Kalia A.N. 2009)**

Herbalists use the leaves, flowers, stems, berries, and roots of plants to prevent, relieve, and treat illness. From a "scientific" perspective, many herbal treatments are considered experimental. The reality is, however, that herbal

medicine has a long and respected history. Many familiar medications of the twentieth century were developed from ancient healing traditions that treated health problems with specific plants. Today, science has isolated the medicinal properties of a large number of botanicals, and their healing components have been extracted and analyzed. Many plant components are now synthesized in large laboratories for use in pharmaceutical preparations. For example, vincristine (an antitumor drug), digitalis (a heart regulator), and ephedrine (a bronchodilator used to decrease respiratory congestion) were all originally discovered through research on plants. **(Agunu A. *et al.,* 2005)**

History of Herbal Medicine

Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. Primitive man observed and appreciated the great diversity of plants available to him. The plants provided food, clothing, shelter, and medicine. Much of the medicinal use of plants seems to have been developed through observations of wild animals, and by trial and error. As time went on, each tribe added the medicinal power of herbs in their area to its knowledgebase. They methodically collected information on herbs and developed well-defined herbal pharmacopoeias. Indeed, well into the 20th century much of the pharmacopoeia of scientific medicine was derived from the herbal lore of native peoples. Many drugs commonly used today are of herbal origin. Indeed, about 25% of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material. Some are made from plant extracts; others are synthesized to mimic a natural plant compound. **(Agus Z.S. *et al.,* 1971)**

Undisputedly, the history of herbology is inextricably intertwined with that of modern medicine. Many drugs listed as conventional medications were originally derived from plants. Salicylic acid, a precursor of aspirin, was originally derived from white willow bark and the meadowsweet plant. Cinchona bark is the source of malaria-fighting quinine. Vincristine, used to treat certain types of cancer, comes from periwinkle. The opium poppy yields morphine, codeine, and paregoric, a treatment for diarrhea Laudanum, a tincture of the opium poppy, was the favored tranquilizer in Victorian times. Even today, morphine-the most important alkaloid of the opium poppy-remains the standard against which new synthetic pain relievers is measured. **(Alder R. *et al.,* 1984)**

- Prior to the discovery and subsequent synthesis of antibiotics, the herb Echinacea (which comes from the plant commonly known as purple coneflower) was one of the most widely prescribed medicines in the United States. For centuries, herbalists prescribed Echinacea to fight infection. Today, research confirms that the herb boosts the immune system by stimulating the production of disease-fighting white blood cells. **(Barbara G. *et al.,* 1986)**

The use of plants as medicine is older than recorded history. As mute witness to this fact marshmallow root, hyacinth, and yarrow have been found carefully tucked around the bones of a Stone Age man in Iraq. These three medicinal herbs continue to be used today. Marshmallow root is a demulcent herb, soothing to inflamed or irritated mucous membranes, such as a sore throat or irritated digestive tract. Hyacinth is a diuretic that encourages tissues to give up excess water. Yarrow is a time-honored cold and fever remedy that may once have been used much as aspirin is today.

In 2735 B.C., the Chinese emperor Shen Nong wrote an authoritative treatise on herbs that is still in use today. Shen Nong recommended the use of Ma Huang (known as ephedra in the Western world), for example, against respiratory distress. Ephedrine, extracted from ephedra, is widely used as a decongestant. You'll find it in its synthetic form, pseudoephedrine, in many allergy, sinus, and cold-relief medications produced by large pharmaceutical companies.

The records of King Hammurabi of Babylon (c. 1800 B.C.) include instructions for using medicinal plants. Hammurabi prescribed the use of mint for digestive disorders. Modern research has confirmed that peppermint does indeed relieve nausea and vomiting by mildly anesthetizing the lining of the stomach.

The entire Middle East has a rich history of herbal healing. There are texts surviving from the ancient cultures of Mesopotamia, Egypt, and India that describe and illustrate the use of many medicinal plant products, including castor oil, linseed oil, and white poppies. In the scriptural book of Ezekiel, which dates from the sixth century B.C., we find this admonition regarding plant life: "..And the fruit thereof shall be for meat, and leaf thereof for medicine." Egyptian hieroglyphs show physicians of the first and second centuries A.D. treating constipation with senna pods, and using caraway and peppermint to relieve digestive upsets. **(Beckette A.H. *et al.,* 1997)**

Throughout the middle Ages, home-grown botanicals were the only medicines readily available, and for centuries, no self-respecting household would be without a carefully tended and extensively used herb garden. For the most part, herbal healing lore was passed from generation to generation by word of mouth. Mother taught daughter; the village herbalist taught a promising apprentice.

By the seventeenth century, the knowledge of herbal medicine was widely disseminated throughout Europe. In 1649, Nicholas Culpeper wrote *A Physical Directory*, and a few years later produced *The English Physician*. This respected herbal pharmacopeia was one of the first manuals that the layperson could use for health care, and it is still widely referred to and quoted today. Culpeper had studied at Cambridge University and was meant to become a great doctor, in the academic sense of the word. Instead, he chose to apprentice to an apothecary and eventually set up his own shop. He served the poor people of London and became known as their neighborhood doctor. The herbal he created was meant for the layperson.

The first U.S. *Pharmacopeia* was published in 1820. This volume included an authoritative listing of herbal drugs, with descriptions of their properties, uses, dosages, and tests of purity. It was periodically revised and became the legal standard for medical compounds in 1906. But as Western medicine evolved from an art to a science in the nineteenth century, information that had at one time been widely available became the domain of comparatively few. Once scientific methods were developed to extract and synthesize the active ingredients in plants, pharmaceutical laboratories took over from providers of medicinal herbs as the producers of drugs. The use of herbs, which for most of history had been mainstream medical practice, began to be considered unscientific, or at least unconventional, and to fall into relative obscurity. **(Berneguer B. *et al.,* 2005)**

Herbal Medicine Today

The World Health Organization (WHO) estimates that 4 billion people, 80% of the world population, presently use herbal medicine for some aspect of primary health care. Herbal medicine is a major component in all indigenous peoples'

traditional medicine and a common element in Ayurvedic, homeopathic, naturopathic, traditional oriental, and Native American Indian medicine. WHO notes that of 119 plant-derived pharmaceutical medicines, about 74% are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures. Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value. **(Bertram G. *et al.,* 2006)**

Today, the U.S. *Pharmacopoeia*, with its reliance on herbal compounds, has been all but forgotten. Most modern physicians rely on the *Physician's Desk Reference*, an extensive listing of chemically manufactured drugs. It is important to note that each entry in this enormous volume, in addition to specifying the chemical compound and actions of a particular drug, also includes an extensive list of contraindications and possible side effects. **(Blandizzi G. *et al.,* 1995)**

Rather than using a whole plant, pharmacologists identify, isolate, extract, and synthesize individual components, thus capturing the active properties. This can create problems, however. In addition to active ingredients, plants contain minerals, vitamins, volatile oils, glycosides, alkaloids, bioflavanoids, and other substances that are important in supporting a particular herb's medicinal properties. These elements also provide an important natural safeguard Isolated or synthesized active compounds can become toxic in relatively small doses; it usually takes a much greater amount of a whole herb, with all of its components, to reach a toxic level. Herbs *are* medicines, however, and they can have powerful effects. They should not tee taken lightly. The suggestions for herbal treatments in this book are not intended

to substitute for consultation with a qualified health care practitioner, but rather to support and assist you in understanding and working with your physician's advice.

Substances derived from the plants remain the basis for a large proportion of the commercial medications used today for the treatment of heart disease, high blood pressure, pain, asthma, and other problems. For example, ephedra is an herb used in Traditional Chinese Medicine for more than two thousand years to treat asthma and other respiratory problems. Ephedrine, the active ingredient in ephedra, is used in the commercial pharmaceutical preparations for the relief of asthma symptoms and other respiratory problems. It helps the patient to breathe more easily.

Another example of the use of an herbal preparation in modern medicine is the foxglove plant. This herb had been in use since 1775. At present, the powdered leaf of this plant is known as the cardiac stimulant digitalis to the millions of heart patients it keeps alive worldwide. **(Bowman W.C. *et al.,* 1980)**

There are over 750,000 plants on earth. Relatively speaking, only a very few of the healing herbs have been studied scientifically. And because modern pharmacology looks for one active ingredient and seeks to isolate it to the exclusion of all the others, most of the research that is done on plants continues to focus on identifying and isolating active ingredients, rather than studying the medicinal properties of whole plants. Herbalists, however, consider that the power of a plant lies in the interaction of all its ingredients. Plants used as medicines offer synergistic interactions between ingredients both known and unknown. **(Canmon D.C. *et al.,* 1969)**

The efficacy of many medicinal plants has been validated by scientists abroad, from Europe to the Orient. Thanks to modern technology, science can now identify some of the specific properties and interactions of botanical constituents. With this scientific documentation, we now know why certain herbs are effective against certain conditions. However, almost all of the current research validating herbal medicine has been done in Germany, Japan, China, Taiwan, and Russia. And for the most part, the United States Food and Drug Administration (FDA), which is responsible for licensing all new drugs (or any substances for which medicinal properties are claimed) for use in the United States, does not recognize or accept findings from across the sea. Doctors and government agencies want to see American scientific studies before recognizing the effectiveness of a plant as medicine. Yet even though substantial research is being done in other countries, drug companies and laboratories in the United States so far have not chosen to put much money or resources into botanical research. The result is that herbal medicine does not have the same place of importance or level of acceptance in this country as it does in other countries. **(Chan F.K.L. *et al.,* 2002)**

Common Herbs and Herbal Preparations

Herbs are available in a variety of forms, including fresh, dried, in tablets or capsules, or bottled in liquid form. You can buy them individually or in mixtures formulated for specific conditions. Whatever type of product you choose, the quality of an herbal preparation-be it in capsule, tablet, tea, tincture, bath, compress, poultice, or ointment form-is only as good as the quality of the raw herb from which it was made. **(Chaturvedi M. *et al.,* 2009)**

Generally, herbs fall into two categories: wild-grown and farm-grown. A wild-grown herb is one that grows naturally, without human intervention. As a result of natural selection, plants tend to be found in places with conditions that optimize their growth. For example, horsetail grows best in moist, swampy areas, while arnica thrives at high altitudes in alpine meadows. The process of gathering herbs from their natural habitats is called *wildcrafting*.

The disadvantage of wild-grown herbs is that there is no guarantee the plants haven't been exposed to chemicals and pesticides. Herbs harvested from a meadow, for example, may have been exposed to chemical drift from a crop-dusted farm nearby. Exhaust fumes from passing traffic may have settled invisibly on plants growing near a country road. Water-loving plants, like horsetail, may be rooted in the bank of a polluted stream.

Because of the possibility of contamination, unless you are very sure of the source of wild crafted herbs, organic herbs grown commercially may be a better choice. Organic farm-grown herbs are becoming increasingly available, as more and more herb farms are being established. With careful management, organic herb farms can provide a steady supply of quality herbs to the consumer.

To produce top-quality products, herb farmers require a great deal of specialized knowledge. For maximum potency, it is important that particular herbs be harvested at the optimum moment. For example, Echinacea is gathered in the spring, winter, and fall, but not in summer, when the plant's energies are concentrated on growth and flowering. **(Coles G.C. *et al.,* 2007)**

Responsible farmers use compost and organic matter to fertilize and replenish the health of the soil. For obvious reasons, we favor the use of certified organically grown herbs, produced without the use of synthetic fertilizers or chemical pesticides. As of this writing, not all states have agencies inspecting and certifying organic growers, so to be sure you are getting pure, pesticide-free herbs grown without chemical contamination, check the label for the words "certified organic" before you make a purchase. The name of the certifying agency should be specified on the label. Two reliable organizations that certify organic products are the Organic Growers and Buyers Association and California Certified Organic Farmers. Organic products grown in the states of Washington and Texas should be certified organic by the Department of Agriculture of the relevant state. As of this writing, federal legislation on requirements for labeling a product "organic" has been passed, but is not yet being fully implemented. Once it is, it should be easier to be sure that you are buying a genuine organic product. Hopefully this will take place in the next few years. **(Dharmani P. *et al.,* 2006)**

Administering Herbal Treatment

Herbs and prepared herbal compounds are available in different forms, each of which has its own particular characteristics. Your health food store will have individual herbs as well as complex herbal formulations, including raw herbs, tinctures, extracts, capsules, tablets, lozenges, and ointments. Here's a look at what's available.

Tinctures

If the label says tincture, the preparation contains alcohol. In a *tincture*, alcohol is employed to extract and concentrate the active properties of the herb. Alcohol is also a very effective natural preservative. Because a tincture is easily assimilated by the body, it is a very effective way to administer herbal compounds. Tinctures are concentrated and cost-effective. However, the full taste of the herb comes through very strongly in a tincture. Children-and adults, too-may find the taste of some herbs unpleasant. Goldenseal, for example, is bitter-tasting.

Another concern when using tinctures is the presence of the alcohol. If you wish to lessen the amount of alcohol in a tincture before giving it to your child, mix the appropriate dose with one-quarter cup of *very* hot water. After about five minutes, most of the taste of the alcohol will have evaporated away, and the mixture should be cool enough to drink. **(Dhivakar M.C. *et al.,* 2008)**

Extracts

Extracts can be made with alcohol, like tinctures, or the essence of the herb can be leached out with water. When purchasing a liquid extract of an herb, the only way to be certain of the extraction process (alcohol or water) is to read the label. Extracts offer essentially the same advantages and disadvantages that tinctures do. They are the most concentrated form of herbal treatment and therefore the most cost-effective. They are easy to administer, but have a strong herbal taste.

Capsules and Tablets

Capsules and tablets contain a ground or powdered form of raw herb. In general, there seems to be little difference between the two in terms of clinical

results. Because finely milled herbs degrade quickly, it is important that herbs be freshly ground and then promptly encapsulated or tableted, within twenty-four hours of being powdered. When making your selection, read the label to make sure fresh herbs have been used in the product. With the exception of certain herbal concentrates in capsule form, both capsules and tablets tend to be much less strong and potent than tinctures and extracts. **(Doll R. *et al.,* 1960)**

Teas

There are many delicious blends of herbal teas on the shelves of your health food store; they need no introduction here. You'll find loose herbs ready for steeping, herbal formulations aimed at specific conditions, and convenient pre-bagged teas. Some are just for sipping; some are medicinal. When your child is ill, a comforting cup of herbal tea (medicinal or not) is a wonderful way to give additional liquids. **(Domanski M. *et. al.,* 2004)**

Lozenges

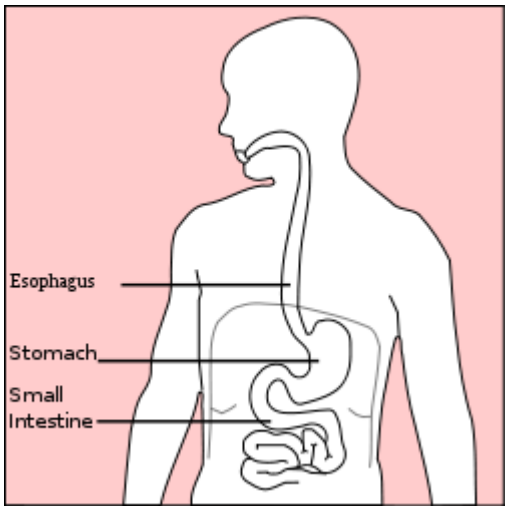
Herbal-based, nutrient-rich, naturally sweetened lozenges are readily available in most health food shops. You'll find cold-fighting formulas, natural cough suppressants, some with decongestant properties. Many are boosted with natural vitamin C. Choose lozenges made without refined sugar.

Ointments, Salves, and Rubs

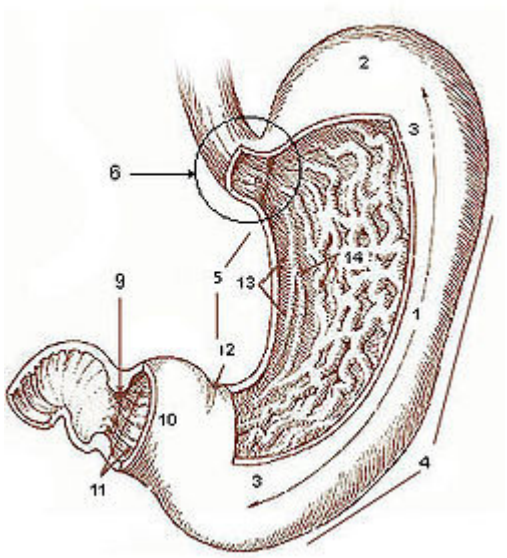
From calendula ointment (for broken skin and wounds) to goldenseal (for infections, rashes, and skin irritations) to aloe vera gel (to cool and speed the healing of minor burns, including sunburn) to heat-producing herbs (for muscle aches and

strains), there's a wealth of topical herbal-based products on the market. Your selection will depend on the condition you are treating. **(Duggan J.M. *et al.*, 2006)**

STOMACH



The location of the stomach in the human body.



Role in digestion

Bolus (masticated food) enters the stomach through the oesophagus via the oesophageal sphincter. The stomach releases proteases (protein-digesting enzymes

such as pepsin) and hydrochloric acid, which kills or inhibits bacteria and provides the acidic pH of two for the proteases to work. Food is churned by the stomach through muscular contractions of the wall – reducing the volume of the fundus, before looping around the fundus and the body of stomach as the boluses are converted into chyme (partially digested food). Chyme slowly passes through the pyloric sphincter and into the duodenum, where the extraction of nutrients begins. Depending on the quantity and contents of the meal, the stomach will digest the food into chyme anywhere between forty minutes and a few hours. **(Bate Smith E.C. *et al.*, 1962)**

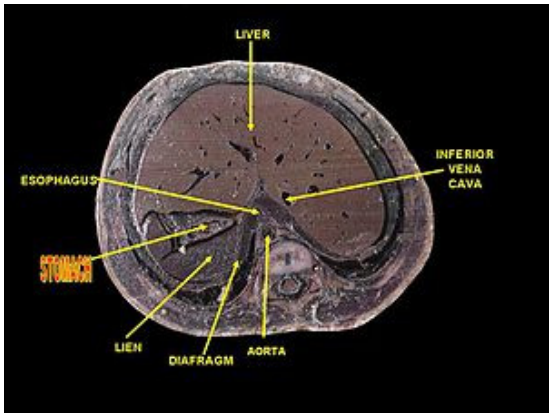
Anatomy of the stomach

The stomach lies between the oesophagus and the duodenum (the first part of the small intestine). It is on the left upper part of the abdominal cavity. The top of the stomach lies against the diaphragm. Lying behind the stomach is the pancreas. The greater momentum hangs down from the *greater curvature*.

Greater omentum and stomach

Two sphincters keep the contents of the stomach contained. They are the esophageal sphincter (found in the cardiac region, not an anatomical sphincter)

dividing the tract above, and the Pyloric sphincter dividing the stomach from the small intestine. **(Elaine N. *et al.,* 2007)**



Stomach

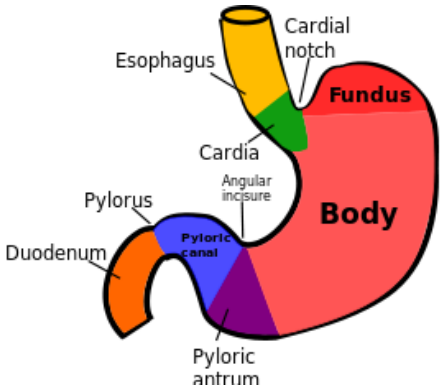
The stomach is surrounded by parasympathetic (stimulant) and orthosympathetic (inhibitor) plexuses (networks of blood vessels and nerves in the anterior gastric, posterior, superior and inferior, celiac and myenteric), which regulate both the secretions activity and the motor (motion) activity of its muscles.

In adult humans, the stomach has a relaxed, near empty volume of about 45 ml. Because it is a distensible organ, it normally expands to hold about one liters of food, but can hold as much as two to three liters. The stomach of a newborn human baby will only be able to retain about 30 ml. **(Ellision DH. *et al.,* 1986)**

Sections

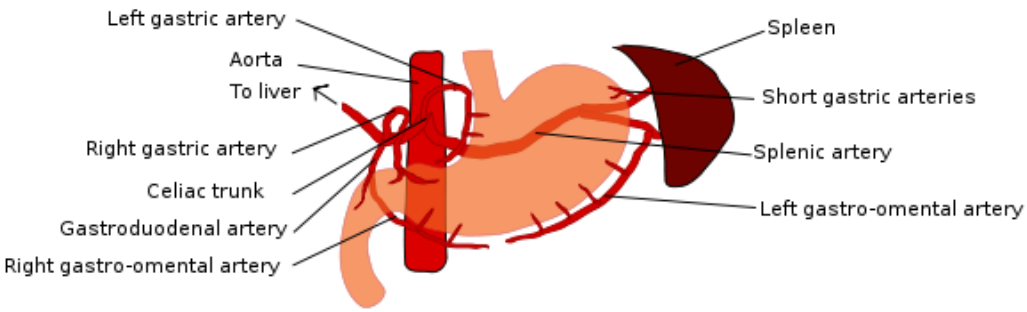
The stomach is divided into four sections, each of which has different cells and functions. The sections are:

Cardia	Where the contents of the oesophagus empty into the stomach.
Fundus	Formed by the upper curvature of the organ.
Body or Corpus	The main, central region.
Pylorus	The lower section of the organ that facilitates emptying the contents into the small intestine.

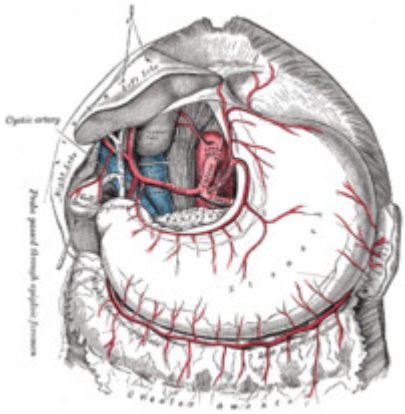


Sections of the stomach

Blood supply



Schematic image of the blood supply to the stomach: left and right gastric artery, left and right gastro-omental artery and short gastric artery. **(Eshaghian *et al.,* 2005)**



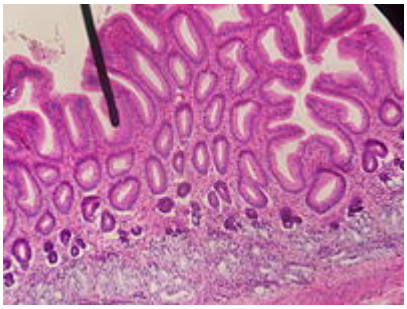
A more realistic image, showing the celiac artery and its branches; the liver has been raised, and the lesser omentum and anterior layer of the greater omentum removed. **(Geert S. *et al.,* 2007)**

The lesser curvature of the stomach is supplied by the right gastric artery inferiorly, and the left gastric artery superiorly, which also supplies the cardiac region. The greater curvature is supplied by the right gastroepiploic artery inferiorly and the left gastroepiploic artery superiorly. The fundus of the stomach, and also the upper portion of the greater curvature, is supplied by the short gastric artery which arises from splenic artery. **(Gooz M. *et al.,* 2001)**

Like the other parts of the gastrointestinal tract, the stomach walls are made of the following layers, from inside to outside:

mucosa	The first main layer. This consists of the epithelium and the lamina propria (composed of loose connective tissue), with a thin layer of smooth muscle called the muscular is mucosae separating it from the submucosa beneath.
submucosa	This layer lies over the mucosa and consists of fibrous connective tissue, separating the mucosa from the next layer. The Meissner's plexus is in this layer.
muscularis externa	Over the submucosa, the muscularis externa in the stomach differs from that of other GI organs in that it has three layers of smooth muscle instead of two. • *inner oblique layer:* This layer is responsible for creating the motion that churns and physically breaks down the food. It is the only layer of the three which is not seen in other parts of the digestive system. The antrum has thicker skin cells in its walls and performs more forceful contractions than the fundus. • *middle circular layer:* At this layer, the pylorus is surrounded by a thick circular muscular wall which is normally topically constricted forming a functional (if not anatomically discrete) pyloric sphincter, which controls the movement of chyme into the duodenum. This layer is concentric to the longitudinal axis of the stomach. • *outer longitudinal layer:* Auerbach's plexus is found between this layer and the middle circular layer.
serosa	This layer is over the muscularis external, consisting of layers of connective tissue continuous with the peritoneum.

Micrograph showing a cross section of the stomach wall, in the body portion of the stomach. H&E stain.

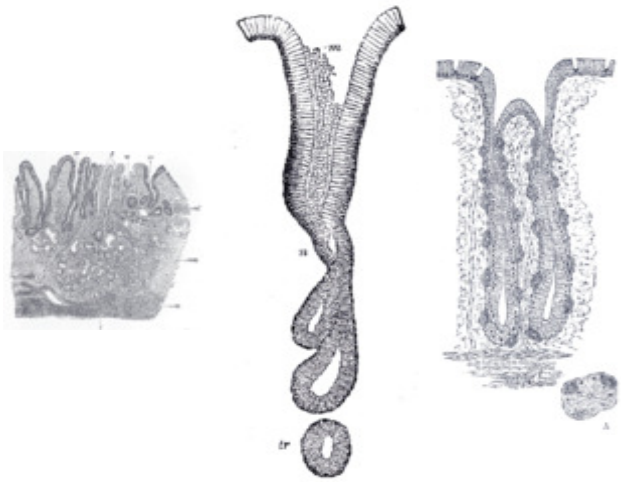


Microscopic cross section of the pyloric part of the stomach wall.

Glands

The epithelium of the stomach forms deep pits. The glands at these locations are named for the corresponding part of the stomach:

Cardiac glands	**Pyloric glands**	**Fundic glands**
(at cardia)	(at pylorus)	(at fundus)



Different types of cells are found at the different layers of these glands:

Layer of stomach	Name	Secretion	Region of stomach	Staining
Isthmus of gland	Mucous neck cells	mucus gel layer	Fundic, cardiac, pyloric	Clear
Body of gland	parietal (oxyntic) cells	gastric acid and intrinsic factor	Fundic only	Acidophilic
Base of gland	chief (zymogenic) cells	pepsinogen	Fundic only	Basophilic
Base of gland	enteroendocrine (APUD) cells	hormones gastrin, histamine, endorphins, serotonin, cholecystokinin and somatostatin	Fundic, cardiac, pyloric	-

Control of secretion and motility

The movement and the flow of chemicals into the stomach are controlled by both the autonomic nervous system and by the various digestive system hormones:

Gastrin	The hormone *gastrin* causes an increase in the secretion of HCl from the parietal cells, and pepsinogen from chief cells in the stomach. It also causes increased motility in the stomach. Gastrin is released by G-cells in the stomach in response to distenstion of the antrum, and digestive products(especially large quantities of incompletely digested proteins). It is inhibited by a pH normally less than 4 (high acid), as well as the hormone somatostatin.

Cholecystokinin	*Cholecystokinin* (CCK) has most effect on the gall bladder, causing gall bladder contractions, but it also decreases gastric emptying and increases release of pancreatic juice which is alkaline and neutralizes the chyme.
Secretin	In a different and rare manner, *secretin*, produced in the small intestine, has most effects on the pancreas, but will also diminish acid secretion in the stomach.
Gastric inhibitory peptide	*Gastric inhibitory peptide* (GIP) decreases both gastric acid release and motility.
Enteroglucagon	*enteroglucagon* decreases both gastric acid and motility.

Other than gastrin, these hormones all act to turn off the stomach action. This is in response to food products in the liver and gall bladder, which have not yet been absorbed. The stomach needs to push food into the small intestine only when the intestine is not busy. While the intestine is full and still digesting food, the stomach acts as storage for food. **(Guptha S. *et al.*, 1996)**

EGF in gastric defense

Epidermal growth factor or EGF results in cellular proliferation, differentiation, and survival. EGF is a low-molecular-weight polypeptide first purified from the mouse submandibular gland, but since then found in many human tissues including submandibular gland, parotid gland. Salivary EGF, which seems also regulated by dietary inorganic iodine, plays also an important physiological role in the maintenance of oro-oesophageal and gastric tissue integrity. The biological effects of salivary EGF include healing of oral and gastroesophageal ulcers,

inhibition of gastric acid secretion, stimulation of DNA synthesis as well as mucosal protection from intraluminal injurious factors such as gastric acid, bile acids, pepsin, and trypsin and to physical, chemical and bacterial agents. **(Guyton *et al.,* 2000)**

Stomach as nutrition sensor

The stomach can "taste" sodium glutamate using glutamate receptors and this information is passed to the lateral hypothalamus and limbic system in the brain as a palatability signal through the vagus nerve. The stomach can also sense independently to tongue and oral taste receptors glucose, carbohydrates proteins, and fats. This allows the brain to link nutritional value of foods to their tastes.

Absorption

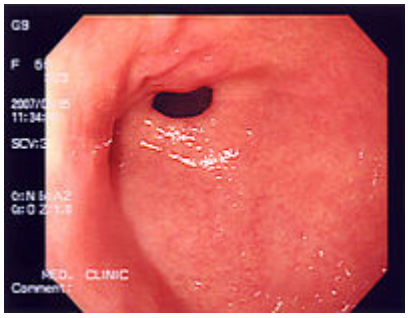
Although the absorption is mainly a function of the small intestine, some absoption of certain small molecules nevertheless does occur in the stomach through its lining. This includes:

- Water, if the body is too dehydrated
- Simple sugars like glucose (e.g. through a glucose drink)
- Medication, like aspirin
- Amino acids (e.g. whey protein shake).

Diseases of the stomach

Historically, it was widely believed that the highly acidic environment of the stomach would keep the stomach immune from infection. However, a large number of studies have indicated that most cases of peptic ulcers, gastritis, and stomach

cancer are caused by *Helicobacter pylori* infection. The stomach has to regenerate a new layer of mucus every two weeks, or else damage to the epithelium may result.



An endoscopy of a normal stomach of a healthy 65-year-old woman.

Although the precise shape and size of the stomach varies widely among different vertebrates, the relative positions of the oesophageal and duodenal openings remain relatively constant. As a result, the organ always curves somewhat to the left before curving back to meet the pyloric sphincter. However, lampreys, hagfishes, chimaeras, lungfishes, and some teleost fish have no stomach at all, with the oesophagus opening directly into the intestine. These animals all consume diets that either require little storage of food, or no pre-digestion with gastric juices, or both. **(Hiruma Lima C.A. *et al.,* 2006)**

The gastric lining is usually divided into two regions, an anterior portion lined by fundic glands, and a posterior with pyloric glands. Cardiac glands are unique to mammals, and even then are absent in a number of species. The distributions of these glands vary between species, and do not always correspond with the same regions as in man. Furthermore, in many non-human mammals, a portion of the stomach anterior to the cardiac glands is lined with epithelium essentially identical to that of the oesophagus. Ruminants, in particular, have a

complex stomach, the first three chambers of which are all lined with oesophageal mucosa. **(Hoogerwerf W.A. *et al.,* 2006)**

In birds and crocodilians, the stomach is divided into two regions. Anteriorly is a narrow tubular region, the proventriculus, lined by fundic glands, and connecting the true stomach to the crop. Beyond lies the powerful muscular gizzard, lined by pyloric glands, and, in some species, containing stones that the animal swallows to help grind up food.

Comparison of stomach glandular regions from several mammalian species. Yellow: oesophagus; green: glandular epithelium; purple: cardiac glands; red: gastric glands; blue: pyloric glands; dark blue: duodenum. Frequency of glands may vary more smoothly between regions than is diagrammed here. Asterisk (ruminant) represents the omasum, which is absent in Tylopoda (Tylopoda also has some cardiac glands opening onto ventral reticulum and nrumen. Many other variations exist among the mammals. **(Lyengar S. *et al.,* 2007)**

Gastric acid

Gastric acid is a digestive fluid, formed in the stomach. It has a pH of 1.5 to 3.5 and is composed of hydrochloric acid (HCl) (around 0.5%, or 5000 parts per million), and large quantities of potassium chloride (KCl) and sodium chloride (NaCl). The acid plays a key role in digestion of proteins, by activating digestive enzymes, and making ingested proteins unravel so that digestive enzymes can break down the long chains of amino acids. **(Niezen J.H. *et al.,* 1995)**

Gastric acid is produced by cells lining the stomach, which are coupled to systems to increase acid production when needed. Other cells in the stomach

produce bicarbonate, a base, to buffer the fluid, ensuring that it does not become too acidic. These cells also produce mucus, which forms a viscous physical barrier to prevent gastric acid from damaging the stomach. Cells in the beginning of the small intestine, or duodenum, further produce large amounts of bicarbonate to completely neutralize any gastric acid that passes further down into the digestive tract.

The presence of gastric acid in the stomach and its function in digestion was first characterized by U.S. Army surgeon William Beaumont around 1830. Beaumont was able to study the stomach action of fur trapper Alexis St. Martin due to the latter's gastric fistula. **(Jeffery *et al.*, 1989)**

Physiology

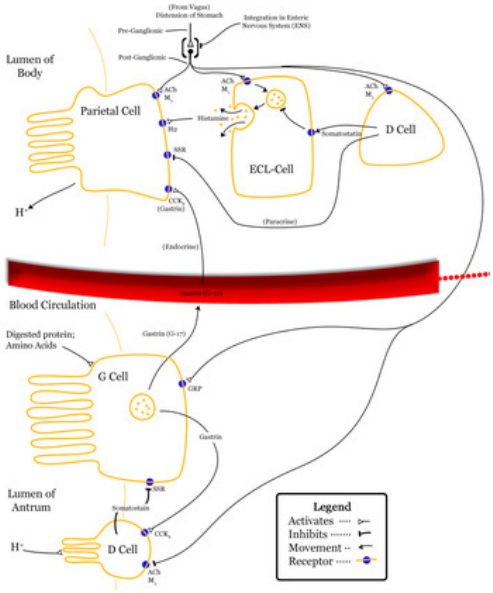


Diagram summarising control of stomach acid secretion, emphasising interaction between the *body* and *antrum*.

Gastric acid is produced by parietal cells (also called oxyntic cells) in the stomach. Its secretion is a complex and relatively energetically expensive process.

Parietal cells contain an extensive secretory network (called canaliculi) from which the gastric acid is secreted into the lumen of the stomach. These cells are part of epithelial fundic glands in the gastric mucosa. The pH of gastric acid is 1.35 to 3.5 in the human stomach lumen, the acidity being maintained by the proton pump H^+/K^+ ATPase. The parietal cell releases bicarbonate into the blood stream in the process, which causes a temporary rise of pH in the blood, known as alkaline tide.

The resulting highly acidic environment in the stomach lumen causes proteins from food to lose their characteristic folded structure (or denature). This exposes the protein's peptide bonds. The chief cells of the stomach secrete enzymes for protein breakdown (inactive pepsinogen and rennin). HCl activates pepsinogen into the enzyme pepsin, which then helps digestion by breaking the bonds linking amino acids, a process known as proteolysis. In addition, many microorganisms have their growth inhibited by such an acidic environment, which is helpful to prevent infection. **(Kannappan N. *et al.,* 2008)**

Secretion

Gastric acid secretion happens in several steps. Chloride and hydrogen ions are secreted separately from the cytoplasm of parietal cells and mixed in the canaliculi. Gastric acid is then secreted into the lumen of the oxyntic gland and gradually reaches the main stomach lumen. The exact manner in which the secreted acid reaches the stomach lumen is controversial, as acid must first cross the relatively pH neutral gastric mucus layer. **(Khandelwal K.R. *et al.,* 2007)**

Chloride and sodium ions are secreted actively from the cytoplasm of the parietal cell into the lumen of the canaliculus. This creates a negative potential of

-40 mV to -70 mV across the parietal cell membrane that causes potassium ions and a small number of sodium ions to diffuse from the cytoplasm into the parietal cell canaliculi.

The enzyme carbonic anhydrase catalyses the reaction between carbon dioxide and water to form carbonic acid. This acid immediately dissociates into hydrogen and bicarbonate ions. The hydrogen ions leave the cell through $H^+/K^\pm$ ATPase antiporter pumps.

At the same time sodium ions are actively reabsorbed. This means that the majority of secreted K^+ and Na^+ ions return to the cytoplasm. In the canaliculus, secreted hydrogen and chloride ions mix and are secreted into the lumen of the oxyntic gland.

The highest concentration that gastric acid reaches in the stomach is 160 mM in the canaliculi. This is about 3 million times that of arterial blood, but almost exactly isotonic with other bodily fluids. The lowest pH of the secreted acid is 0.8, but the acid is diluted in the stomach lumen to a pH between 1 and 3.

There are three phases in the secretion of gastric acid:

1. The cephalic phase: Thirty percent of the total gastric acid secretions to be produced are stimulated by anticipation of eating and the smell or taste of food.

2. The gastric phase: Sixty percent of the acid secreted is stimulated by the distention of the stomach with food. Plus, digestion produces proteins, which causes even more gastrin production.

3. The intestinal phase: The remaining 10% of acid is secreted when chyme enters the small intestine, and is stimulated by small intestine distention.

There is also a small continuous basal secretion of gastric acid between meals of usually less than 10 mEq/hour.

Regulation of secretion

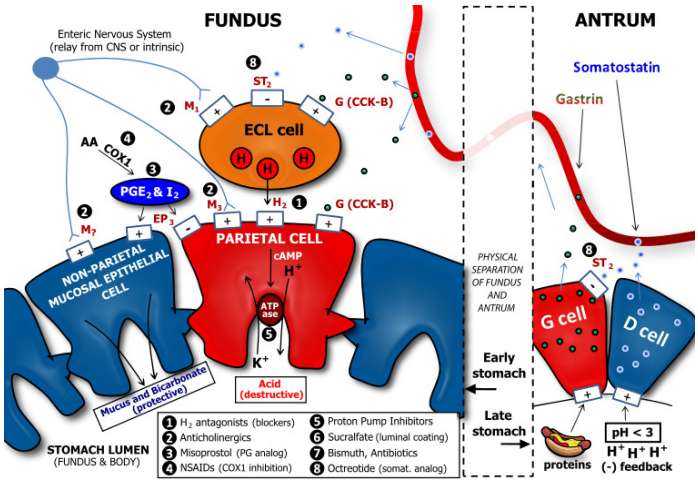


Diagram depicting the major determinants of gastric acid secretion, with inclusion of drug targets for peptic ulcer disease (PUD) and gastroesophageal reflux disease (GERD).

Gastric acid production is regulated by both the autonomic nervous system and several hormones. The parasympathetic nervous system, via the vagus nerve, and the hormone gastrin stimulate the parietal cell to produce gastric acid, both directly acting on parietal cells and indirectly, through the stimulation of the secretion of the hormone histamine from enterochromaffine-like cells (ECL). Vasoactive intestinal peptide, cholecystokinin, and secretin all inhibit production.

The production of gastric acid in the stomach is tightly regulated by positive regulators and negative feedback mechanisms. Four types of cells are involved in

this process: parietal cells, G cells, D cells and enterochromaffine-like cells. Besides this, the endings of the vagus nerve (CN X) and the intramural nervous plexus in the digestive tract influence the secretion significantly. **(Kokate C.K. 1985)**

Nerve endings in the stomach secrete two stimulatory neurotransmitters: acetylcholine and gastrin-releasing peptide. Their action is both direct on parietal cells and mediated through the secretion of gastrin from G cells and histamine from enterochromaffine-like cells. Gastrin acts on parietal cells directly and indirectly too, by stimulating the release of histamine. **(Malairajan. P. *et al.,* 2007)**

The release of histamine is the most important positive regulation mechanism of the secretion of gastric acid in the stomach. Its release is stimulated by gastrin and acetylcholine and inhibited by somatostatin.

Neutralization

In the duodenum, gastric acid is neutralized by sodium bicarbonate. This also blocks gastric enzymes that have their optima in the acid range of pH. The secretion of sodium bicarbonate from the pancreas is stimulated by secretin. This polypeptide hormone gets activated and secreted from so-called S cells in the mucosa of the duodenum and jejunum when the pH in duodenum falls below 4.5 to 5.0. The neutralization is described by the equation:

$$HCl + NaHCO_3 \rightarrow NaCl + H_2CO_3$$

The carbonic acid instantly decomposes into carbon dioxide and water.

Role in disease

In hypochlorhydria and achlorhydria, there is low or no gastric acid in the stomach, potentially leading to problems as the disinfectant properties of the gastric lumen are decreased. In such conditions, there is greater risk of infections of the digestive tract (such as infection with *Vibrio* or *Helicobacter* bacteria). **(Mozafar Khazaei *et al.,* 2006)**

In Zollinger-Ellison syndrome and hypercalcemia, there are increased gastrin levels, leading to excess gastric acid production, which can cause gastric ulcers.

In diseases featuring excess vomiting, patients develop hypochloremic metabolic alkalosis (decreased blood acidity by H^+ and chlorine depletion).

Pharmacology

The proton pump enzyme is the target of proton pump inhibitors, used to increase gastric pH in diseases that feature excess acid. H_2 antagonists indirectly decrease gastric acid production. Antacids neutralize existing acid.

The Parietal Cell: Mechanism of Acid Secretion

The best-known component of gastric juice is hydrochloric acid, the secretory product of the parietal, or oxyntic cell. It is known that the capacity of the stomach to secrete HCl is almost linearly related to parietal cell numbers.

When stimulated, parietal cells secrete HCl at a concentration of roughly 160 mM (equivalent to a pH of 0.8). The acid is secreted into large cannaliculi, deep invaginations of the plasma membrane which are continuous with the lumen of the stomach.

When acid secretion is stimulated there is a dramatic change in the morphology of the membranes of the parietal cell. Cytoplasmic tubulovesicular membranes which are abundant in the resting cell virtually disappear in concert with a large increase in the cannalicular membrane. It appears that the proton pump as well as potassium and chloride conductance channels initially reside on intracellular membranes and are transported to and fused into the cannalicular membrane just prior to acid secretion. **(Nelson D.L. *et al.,* 2005)**

The epithelium of the stomach is intrinsically resistant to the damaging effects of gastric acid and other insults. Nonetheless, excessive secretion of gastric acid is a major problem in human and, to a lesser extent, animal populations, leading to gastritis, gastric ulcers and peptic acid disease. As a consequence, the parietal cell and the mechanisms it uses to secrete acid have been studied extensively, leading to development of several drugs useful for suppressing acid secretion. **(Oates P.J. *et al.,* 1988)**

Mechanism of Acid Secretion

The hydrogen ion concentration in parietal cell secretions is roughly 3 million fold higher than in blood, and chloride is secreted against both a concentration and electric gradient. Thus, the ability of the partietal cell to secrete acid is dependent on active transport.

The key player in acid secretion is a H+/K+ ATPase or "proton pump" located in the cannalicular membrane. This ATPase is magnesium-dependent, and not inhibitable by ouabain. The current model for explaining acid secretion is as follows:

- Hydrogen ions are generated within the parietal cell from dissociation of water. The hydroxyl ions formed in this process rapidly combine with carbon dioxide to form bicarbonate ion, a reaction cataylzed by carbonic anhydrase.

- Bicarbonate is transported out of the basolateral membrane in exchange for chloride. The outflow of bicarbonate into blood results in a slight elevation of blood pH known as the "alkaline tide". This process serves to maintain intracellular pH in the parietal cell.

- Chloride and potassium ions are transported into the lumen of the cannaliculus by conductance channels, and such is necessary for secretion of acid.

- Hydrogen ion is pumped out of the cell, into the lumen, in exchange for potassium through the action of the proton pump; potassium is thus effectively recycled.

- Accumulation of osmotically-active hydrogen ion in the cannaliculus generates an osmotic gradient across the membrane that results in outward diffusion of water - the resulting gastric juice is 155 mM HCl and 15 mM KCl with a small amount of NaCl.

These processes are depicted in the animation below.

Control of Acid Secretion

Parietal cells bear receptors for three stimulators of acid secretion, reflecting a triumverate of neural, paracrine and endocrine control:

- **Acetylcholine** (muscarinic type receptor)
- **Gastrin**
- **Histamine** (H_2 type receptor)

Histamine from enterochromaffin-like cells may well be the primary modulator, but the magnitude of the stimulus appears to result from a complex additive or multiplicative interaction of signals of each type. For example, the low amounts of histamine released constantly from mast cells in the gastric mucosa only weakly stimulate acid secretion, and similarly for low levels of gastrin or acetylcholine. However, when low levels of each are present, acid secretion is strongly forced. Additionally, pharmacologic antagonists of each of these molecules can block acid secretion. **(Patil K.S. *et al.,* 2008)**

Histamine's effect on the parietal cell is to activate adenylate cyclase, leading to elevation of intracellular cyclic AMP concentrations and activation of protein kinase A (PKA). One effect of PKA activation is phosphorylation of cytoskeletal proteins involved in transport of the H+/K+ ATPase from cytoplasm to plasma membrane.

Binding of acetylcholine and gastrin both result in elevation of intracellular calcium concentrations.

The animation below depicts acid secretion by the parietal cell. Even though many of the actors are unlabeled, you should be able to deduce the identity of all the components you see.

Several additional mediators have been shown to result in gastric acid secretion when infused into animals and people, including calcium, enkephalin and bombesin. Calcium and bombesin both simulate gastrin release, while opiate receptors have been identified on parietal cells. It is unclear whether these molecules have a significant physiologic role in parietal cell function. **(Paul V. *et al.,* 2000)**

A variety of substances are capable of reducing gastric acid secretion when infused intravenously, including prostaglandin E_2 and several peptides hormones, including secretin, gastric inhibitory peptide, glucagon and somatostatin. PGE_2, secretin and somatostatin may be physiologic regulators. Somatostatin inhibits secretion of gastrin and histamine, and appears to have a direct inhibitory effect on the parietal cell.

PEPTIC ULCER

Classification and external resources

Deep gastric ulcer

A **peptic ulcer**, also known as **PUD** or **peptic ulcer disease**, is the most common ulcer of an area of the gastrointestinal tract that is usually acidic and thus extremely painful. It is defined as mucosal erosions equal to or greater than 0.5 cm. As many as 70–90% of such ulcers are associated with *Helicobacter pylori*, a spiral-shaped bacterium that lives in the acidic environment of the stomach; however, only 40% of those cases go to a doctor. Ulcers can also be caused or worsened by drugs such as aspirin, ibuprofen, and other NSAIDs. **(Rajkapoor B. *et al.,* 2002)**

Four times as many peptic ulcers arise in the duodenum—the first part of the small intestine, just after the stomach—as in the stomach itself. About 4% of gastric ulcers are caused by a malignant tumor, so multiple biopsies are needed to exclude cancer. Duodenal ulcers are generally benign. **(Rang H.B. 2003)**

Classification

By Region/Location

- Duodenum (called duodenal ulcer)
- Oesophagus (called esophageal ulcer)
- Stomach (called gastric ulcer)
- Meckel's diverticulum (called Meckel's diverticulum ulcer; is very tender with palpation)

Modified Johnson Classification of peptic ulcers:

- Type **I**: Ulcer along the body of the stomach, most often along the lesser curve at incisura angularis along the locus minoris resistentiae.
- Type **II**: Ulcer in the body in combination with duodenal ulcers. Associated with acid oversecretion.
- Type **III**: In the pyloric channel within 3 cm of pylorus. Associated with acid oversecretion.
- Type **IV**: Proximal gastroesophageal ulcer
- Type **V**: Can occur throughout the stomach. Associated with chronic NSAID use (such as aspirin).

Signs and symptoms

Symptoms of a peptic ulcer can be

- abdominal pain, classically epigastric with severity relating to mealtimes, after around three hours of taking a meal (duodenal ulcers are classically relieved by food, while gastric ulcers are exacerbated by it)

- bloating and abdominal fullness
- waterbrash (rush of saliva after an episode of regurgitation to dilute the acid in esophagus - although this is more associated with gastro-esophageal reflux disease)
- nausea, and copious vomiting
- loss of appetite and weight loss;
- Hematemesis (vomiting of blood); this can occur due to bleeding directly from a gastric ulcer, or from damage to the esophagus from severe/continuing vomiting.
- melena (tarry, foul-smelling feces due to oxidized iron from hemoglobin);
- Rarely, an ulcer can lead to a gastric or duodenal perforation, which leads to acute peritonitis. This is extremely painful and requires immediate surgery.

A history of heartburn, gastro-esophageal reflux disease (GERD) and use of certain forms of medication can raise the suspicion for peptic ulcer. Medicines associated with peptic ulcer include NSAID (non-steroid anti-inflammatory drugs) that inhibit cyclooxygenase, and most glucocorticoids (e.g. dexamethasone and prednisolone). **(Rao C.V. *et al.*, 2004)**

In patients over 45 with more than two weeks of the above symptoms, the odds for peptic ulceration are high enough to warrant rapid investigation by esophagogastroduodenoscopy.

The timing of the symptoms in relation to the meal may differentiate between gastric and duodenal ulcers: A gastric ulcer would give epigastric pain during the meal, as gastric acid production is increased as food enters the stomach. Symptoms of duodenal ulcers would initially be relieved by a meal, as the pyloric sphincter closes to concentrate the stomach contents, therefore acid is not reaching the duodenum. Duodenal ulcer pain would manifest mostly 2–3 hours after the meal, when the stomach begins to release digested food and acid into the duodenum.

Also, the symptoms of peptic ulcers may vary with the location of the ulcer and the patient's age. Furthermore, typical ulcers tend to heal and recur and as a result the pain may occur for few days and weeks and then wane or disappear. Usually, children and the elderly do not develop any symptoms unless complications have arisen. **(Ruediger Gay *et al.,* 1984)**

Burning or gnawing feeling in the stomach area lasting between 30 minutes and 3 hours commonly accompanies ulcers. This pain can be misinterpreted as hunger, indigestion or heartburn. Pain is usually caused by the ulcer but it may be aggravated by the stomach acid when it comes into contact with the ulcerated area. The pain caused by peptic ulcers can be felt anywhere from the navel up to the sternum, it may last from few minutes to several hours and it may be worse when the stomach is empty. Also, sometimes the pain may flare at night and it can commonly be temporarily relieved by eating foods that buffer stomach acid or by taking anti-acid medication. However, peptic ulcer disease symptoms may be different for every sufferer. **(Savioli L. *et al.,* 2002)**

Complications

- Gastrointestinal bleeding is the most common complication. Sudden large bleeding can be life-threatening. It occurs when the ulcer erodes one of the blood vessels, such as the gastroduodenal artery.

- Perforation (a hole in the wall) often leads to catastrophic consequences. Erosion of the gastro-intestinal wall by the ulcer leads to spillage of stomach or intestinal content into the abdominal cavity. Perforation at the anterior surface of the stomach leads to acute peritonitis, initially chemical and later bacterial peritonitis. The first sign is often sudden intense abdominal pain. Posterior wall perforation leads to bleeding due to involvement of gastro-duodenal artery that lies posterior to the 1st part of duodenum.

- Penetration is when the ulcer continues into adjacent organs such as the liver and pancreas.

- Scarring and swelling due to ulcers causes narrowing in the duodenum and gastric outlet obstruction. Patient often presents with severe vomiting.

- Cancer is included in the differential diagnosis (elucidated by biopsy), Helicobacter pylori as the etiological factor making it 3 to 6 times more likely to develop stomach cancer from the ulcer.

Cause

A major causative factor (60% of gastric and up to 90% of duodenal ulcers) is chronic inflammation due to *Helicobacter pylori* that colonizes the antral mucosa. The immune system is unable to clear the infection, despite the appearance of antibodies. Thus, the bacterium can cause a chronic active gastritis (type B gastritis),

resulting in a defect in the regulation of gastrin production by that part of the stomach, and gastrin secretion can either be increased, or as in most cases, decreased, resulting in hypo- or achlorhydria. Gastrin stimulates the production of gastric acid by parietal cells and, in *H. pylori* colonization responses that increase gastrin, the increase in acid can contribute to the erosion of the mucosa and therefore ulcer formation.

Another major cause is the use of NSAIDs. The gastric mucosa protects itself from gastric acid with a layer of mucus, the secretion of which is stimulated by certain prostaglandins. NSAIDs block the function of cyclooxygenase 1 (*cox-1*), which is essential for the production of these prostaglandins. COX-2 selective anti-inflammatories (such as celecoxib or the since withdrawn rofecoxib) preferentially inhibit *cox-2*, which is less essential in the gastric mucosa, and roughly halve the risk of NSAID-related gastric ulceration. As the prevalence of *H. pylori*-caused ulceration declines in the Western world due to increased medical treatment, a greater proportion of ulcers will be due to increasing NSAID use among individuals with pain syndromes as well as the growth of aging populations that develop arthritis.

The incidence of duodenal ulcers has dropped significantly during the last 30 years, while the incidence of gastric ulcers has shown a small increase, mainly caused by the widespread use of NSAIDs. The drop in incidence is considered to be a cohort-phenomenon independent of the progress in treatment of the disease. The cohort-phenomenon is probably explained by improved standards of living which has lowered the incidence of *H. pylori* infections.

Although some studies have found correlations between smoking and ulcer formation, others have been more specific in exploring the risks involved and have found that smoking by itself may not be much of a risk factor unless associated with *H. pylori* infection. Some suggested risk factors such as diet, spice consumption and blood type, were hypothesized as ulcerogens (helping cause ulcers) until late in the 20th century, but have been shown to be of relatively minor importance in the development of peptic ulcers. Similarly, while studies have found that alcohol consumption increases risk when associated with *H. pylori* infection, it does not seem to independently increase risk, and even when coupled with *H. pylori* infection, the increase is modest in comparison to the primary risk factor.

Gastrinomas (Zollinger Ellison syndrome), rare gastrin-secreting tumors, also cause multiple and difficult-to-heal ulcers.

Stress

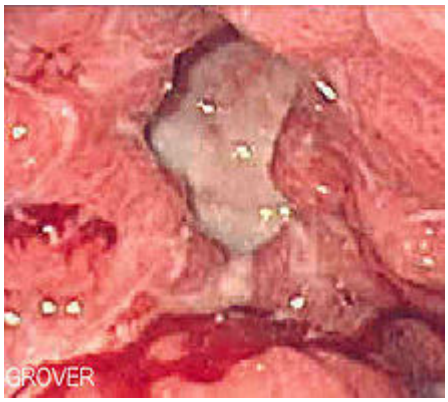
Researchers also continue to look at stress as a possible cause, or at least complication, in the development of ulcers. There is debate as to whether psychological stress can influence the development of peptic ulcers. Burns and head trauma, however, can lead to physiologic stress ulcers, which are reported in many patients who are on mechanical ventilation.

An expert panel convened by the Academy of Behavioral Medicine Research concluded that ulcers are not purely an infectious disease and that psychological factors do play a significant role. Researchers are examining how stress might promote *H. pylori* infection. For example, *Helicobacter pylori* thrive in an acidic environment, and stress has been demonstrated to cause the production of excess

stomach acid. This was supported by a study on mice showing that both long-term water-immersion-restraint stress and *H. pylori* infection were independently associated with the development of peptic ulcers.

A study of peptic ulcer patients in a Thai hospital showed that chronic stress was strongly associated with an increased risk of peptic ulcer, and a combination of chronic stress and irregular mealtimes was a significant risk factor.

Diagnosis



Endoscopic image of gastric ulcer, biopsy proven to be gastric cancer.

The diagnosis is mainly established based on the characteristic symptoms. Stomach pain is usually the first signal of a peptic ulcer. In some cases, doctors may treat ulcers without diagnosing them with specific tests and observe whether the symptoms resolve, this indicating that their primary diagnosis was accurate.

Confirmation of the diagnosis is made with the help of tests such as endoscopies or barium contrast x-rays. The tests are typically ordered if the symptoms do not resolve after a few weeks of treatment, or when they first appear in a person who is over age 45 or who has other symptoms such as weight loss, because stomach cancer can cause similar symptoms. Also, when severe ulcers resist

treatment, particularly if a person has several ulcers or the ulcers are in unusual places, a doctor may suspect an underlying condition that causes the stomach to overproduce acid.

An esophagogastroduodenoscopy (EGD), a form of endoscopy, also known as a gastroscopy, is carried out on patients in whom a peptic ulcer is suspected. By direct visual identification, the location and severity of an ulcer can be described. Moreover, if no ulcer is present, EGD can often provide an alternative diagnosis.

One of the reasons that blood tests are not reliable for accurate peptic ulcer diagnosis on their own is their inability to differentiate between past exposure to the bacteria and current infection. Additionally, a false negative result is possible with a blood test if the patient has recently been taking certain drugs, such as antibiotics or proton pump inhibitors.

The diagnosis of *Helicobacter pylori* can be made by:

- Urea breath test (noninvasive and does not require EGD)
- Direct culture from an EGD biopsy specimen; this is difficult to do, and can be expensive. Most labs are not set up to perform *H. pylori* cultures
- Direct detection of urease activity in a biopsy specimen by rapid urease test
- Measurement of antibody levels in blood (does not require EGD). It is still somewhat controversial whether a positive antibody without EGD is enough to warrant eradication therapy
- Stool antigen test

- Histological examination and staining of an EGD biopsy.

The breath test uses radioactive carbon atom to detect H. pylori. To perform this exam the patient will be asked to drink a tasteless liquid which contains the carbon as part of the substance that the bacteria breaks down. After an hour, the patient will be asked to blow into a bag that is sealed. If the patient is infected with H. pylori, the breath sample will contain radioactive carbon dioxide. This test provides the advantage of being able to monitor the response to treatment used to kill the bacteria.

The possibility of other causes of ulcers, notably malignancy (gastric cancer) needs to be kept in mind. This is especially true in ulcers of the *greater (large) curvature* of the stomach; most are also a consequence of chronic *H. pylori* infection.

If a peptic ulcer perforates, air will leak from the inside of the gastrointestinal tract (which always contains some air) to the peritoneal cavity (which normally never contains air). This leads to "free gas" within the peritoneal cavity. If the patient stands erect, as when having a chest X-ray, the gas will float to a position underneath the diaphragm. Therefore, gas in the peritoneal cavity, shown on an erect chest X-ray or supine lateral abdominal X-ray, is an omen of perforated peptic ulcer disease.

Macroscopic appearance

A benign gastric ulcer (from the antrum) of a gastrectomy specimen.

Gastric ulcers are most often localized on the lesser curvature of the stomach. The ulcer is a round to oval parietal defect ("hole"), 2 to 4 cm diameter, with a smooth base and perpendicular borders. These borders are not elevated or irregular in the acute form of peptic ulcer, regular but with elevated borders and inflammatory surrounding in the chronic form. In the ulcerative form of gastric cancer the borders are irregular. Surrounding mucosa may present radial folds, as a consequence of the parietal scarring.

Microscopic appearance

A gastric peptic ulcer is a mucosal defect which penetrates the muscularis mucosae and muscularis propria, produced by acid-pepsin aggression. Ulcer margins are perpendicular and present chronic gastritis. During the active phase, the base of the ulcer shows 4 zones: inflammatory exudate, fibrinoid necrosis, granulation tissue and fibrous tissue. The fibrous base of the ulcer may contain vessels with thickened wall or with thrombosis.

Differential diagnosis

- Peptic ulcer
- Gastritis

- Stomach cancer
- Gastroesophageal reflux disease
- Pancreatitis
- Hepatic congestion
- Cholecystitis
- Biliary colic
- Inferior myocardial infarction
- Referred pain (pleurisy, pericarditis)
- Superior mesenteric artery syndrome

Treatment

Younger patients with ulcer-like symptoms are often treated with antacids or H2 antagonists before EGD is undertaken. Bismuth compounds may actually reduce or even clear organisms, though the warning labels of some bismuth subsalicylate products indicate that the product should not be used by someone with an ulcer.

Patients who are taking non-steroidal anti-inflammatories (NSAIDs) may also be prescribed a prostaglandin analogue (Misoprostol) in order to help prevent peptic ulcers, which may be a side-effect of the NSAIDs.

When *H. pylori* infection is present, the most effective treatments are combinations of two antibiotics (e.g. Clarithromycin, Amoxicillin, Tetracycline, Metronidazole) and 1 proton pump inhibitor (PPI), sometimes together with a bismuth compound. In complicated, treatment-resistant cases, 3 antibiotics (e.g. amoxicillin + clarithromycin + metronidazole) may be used together with a PPI and sometimes with bismuth compound. An effective first-line therapy for

uncomplicated cases would be Amoxicillin + Metronidazole + Pantoprazole (a PPI). In the absence of *H. pylori*, long-term higher doses of PPIs are often used.

Treatment of *H. pylori* usually leads to clearing of infection, relief of symptoms and eventual healing of ulcers. Recurrence of infection can occur and retreatment may be required, if necessary with other antibiotics. Since the widespread use of PPI's in the 1990s, surgical procedures (like "highly selective vagotomy") for uncomplicated peptic ulcers became obsolete.

Perforated peptic ulcer is a surgical emergency and requires surgical repair of the perforation. Most bleeding ulcers require endoscopy urgently to stop bleeding with cautery, injection, or clipping.

Ranitidine provides relief of peptic ulcers, heartburn, indigestion and excess stomach acid and prevention of these symptoms associated with excessive consumption of food and drink. Ranitidine is available over the counter from a pharmacy and works by decreasing the amount of acid the stomach produces allowing healing of ulcers. Zantac tablets contain Ranitidine 150 mg as the active ingredient which can also be bought generically.

Sucralfate, (Carafate) has also been a successful treatment of peptic ulcers.

Epidemiology

The lifetime risk for developing a peptic ulcer is approximately 10%.

In Western countries the prevalence of *Helicobacter pylori* infections roughly matches age (i.e., 20% at age 20, 30% at age 30, 80% at age 80 etc.). Prevalence is higher in third world countries. Transmission is by food, contaminated

groundwater, and through human saliva (such as from kissing or sharing food utensils).

A minority of cases of *H. pylori* infection will eventually lead to an ulcer and a larger proportion of people will get non-specific discomfort, abdominal pain or gastritis.

Peptic ulcer disease had a tremendous effect on morbidity and mortality until the last decades of the 20th century, when epidemiological trends started to point to an impressive fall in its incidence. The reason that the rates of peptic ulcer disease decreased is thought to be the development of new effective medication and acid suppressants and the discovery of the cause of the condition, *H. pylori*.

In the United States about 4 million people have active peptic ulcers and about 350,000 new cases are diagnosed each year. Four times as many duodenal ulcers as gastric ulcers are diagnosed. Approximately 3,000 deaths per year in the United States are due to duodenal ulcer and 3,000 to gastric ulcer.

History

John Lykoudis, a general practitioner in Greece, treated patients for peptic ulcer disease with antibiotics, beginning in 1958, long before it was commonly recognized that bacteria were a dominant cause for the disease.

Helicobacter pylori were rediscovered in 1982 by two Australian scientists, Robin Warren and Barry J. Marshall as a causative factor for ulcers. In their original paper, Warren and Marshall contended that most gastric ulcers and gastritis were caused by colonization with this bacterium, not by stress or spicy food as had been assumed before.

The *H. pylori* hypothesis was poorly received, so in an act of self-experimentation Marshall drank a Petri dish containing a culture of organisms extracted from a patient and five days later developed gastritis. His symptoms disappeared after two weeks, but he took antibiotics to kill the remaining bacteria at the urging of his wife, since halitosis is one of the symptoms of infection. This experiment was published in 1984 in the Australian Medical Journal and is among the most cited articles from the journal.

In 1997, the Centers for Disease Control and Prevention, with other government agencies, academic institutions, and industry, launched a national education campaign to inform health care providers and consumers about the link between *H. pylori* and ulcers. This campaign reinforced the news that ulcers are a curable infection, and that health can be greatly improved and money saved by disseminating information about *H. pylori*.

In 2005, the Karolinska Institute in Stockholm awarded the Nobel Prize in Physiology or Medicine to Dr. Marshall and his long-time collaborator Dr. Warren "for their discovery of the bacterium *Helicobacter pylori* and its role in gastritis and peptic ulcer disease." Professor Marshall continues research related to *H. pylori* and runs a molecular biology lab at UWA in Perth, Western Australia.

Some believed that mastic gum, a tree resin extract, actively eliminates the *H. pylori* bacteria. However, multiple subsequent studies have found no effect of using mastic gum on reducing *H. pylori* levels.

2. LITERATURE REVIEW

Literature review is the first and most important step for the proper selection of plants and it also forms basis for the planning of any scientific work that is to be performed. There is no study done on the plant fruit part of the *Ficus arnottiana Miq*. Review literature about the different parts of the *Ficus arnottiana Miq* done under various divisions like pharmacological, hypoglycemic, cardio protective activity, protective effects on testicular effects, medical and also miscellaneous reviews.

Papiya Mitra Mazumder *et.al.* has reported on" HYPOGLYCEMIC AND ANTIOXIDANT ACTIVITY OF AN ISOLATED COMPOUND FROM Ficus arnottiana Miq bark The hypoglycemic and antioxidant effect of an isolated compound (Ficanone) from *Ficus arnottiana Miq* bark was investigated in normal and streptozotocin- diabetic rats. Administration of petroleum ether, chloroform, acetone and methanol extracts of Ficus arnottiana Miq bark at a dose of 100 mg/kg, p.o. for 21 days caused a decrease in fasting blood sugar in diabetic rats (FBS). Among all the extracts, acetone extract was found to lower the FBS significantly in diabetic rats. In acute oral toxicity studies (OECD-425 guidelines), no mortality was observed up to the highest dose of acetone extract (2000 mg/kg, p.o). Acetone extract of the bark was subjected to column chromatography and Ficanone was isolated. Phytochemical studies indicated that Ficanone is a triterpenoidal compound. When administered to diabetic rats, Ficanone (50 mg/kg, p.o.) caused a significant ($p<0.01$) reduction in FBS. Ficanone also caused a considerable decrease in lipid peroxidation and improvement in the antioxidant enzymes (reduced glutathione, superoxide dismutase and catalase) levels in diabetic rats.

Histopathology of pancreas also indicated improvement in the condition of β cells after treatment with Ficanone. The results of the present study indicated that Ficanone has antioxidant potential with antidiabetic activity and provides a scientific rationale for the use of Ficanone as an antidiabetic agent.

*__M. Jhansi Rani__, ***et.al*** Peptic Ulcer disease is a serious gastrointestinal disorder that requires a well-targeted therapeutic strategy. A number of drug including proton pump inhibitor and H2 receptor antagonists an available treatment of peptic ulcer. The present article review the anti ulcerogenic ulcer healing property Garcinia cambogia, Terminalia chebula, Napoleona vogelii, polythia longifolia, allophylus serratus kurz, Dodonaea viscose, some of the important medicinal plants reported for their anti ulcer and ulcer healing property. The pathophysiology of peptic ulcer disorder involves an imbalance between offensive (Acid, pepsion and H. Pylori) and defensive factors (Mucin prostaglandin, bicarbonate, nitric oxide growth factor. Indian medicinal plants and their derivatives have been an invaluable source of therapeutic agents to treat various disorder include peptic ulcer. Despite progress in conventional chemistry and pharmacology producing effective drugs the plant kindom useful source of new anti-ulcer compounds for development of pharmaceutical entities.

Saha rajasekar et.al., has reported on Analgesic activity of *Ficus arnottina* Miq. Leaves extract the methanolic extract leaves of Ficus arnottina was used to evaluate the Analgesic activity. The above activity was evaluated using the eddy's hot plate and heat conduction method and acetic acid induced writing in mice in mice. The dose used for the test of activity (100, 200, 400 mg/kg ip). The extract at all doses tested significantly ($p<0.001$) inhibited acetic induced writhing and also

significantly ($p<0.05$) prolonged the reaction latency to pain thermally induced in mice by the hot plate. The phytochemical screening revealed the presence of alkaloids flavonoids glycosides saponins tannins which might be responsible for the observed analgesic and anti-inflammatory activity. This study showed that Ficus arnottina possess significant anti-inflammatory and analgesic properties in rodents which supported the folkloric claim for the use of the plant as a medicine.

Ramandeep Singh et.al.,

Many diseases are associated with oxidative stress caused by free radicals. The Present research was carried out to evaluate in vitro antioxidant activity potential by five different methods of various extracts of bark of Ficus arnottiana Miq. Antioxidant activity was determined by using five different in vitro assay including total phenolic content (TPC), Total reducing power, DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging, Total flavonoid content and Hydroxyl ion Scavenging assay. The decreasing order of antioxidant activities is acetone extract (FAAE)>Methanol extract (FAME)>petroleum ether extract (FAPEE)>chloroform extract (FACE) in all the methods which is in conformity with TPC. The results clearly demonstrate that acetone extract has highest TPC and displayed strongest activity, and can be used to prevent oxidative stress related diseases. The processing of perishable bark of Ficus arnottiana Miq .by selective extraction with acetone can give better yield of antioxidants and the extract can be stored as food supplement with longer shelf life. Further investigation of individual isolated compounds and their in vivo antioxidant activities and in different antioxidant mechanisms is needed.

3. AIM AND PLAN OF WORK

It is estimated by world health organization that, 80% of the world population must rely on traditional medicines for health care; these traditional medicines are mainly plant based. Most of the studies demonstrate the importance of natural products in drug discovery. The use of phytoconstituents as drug therapy to treat major ailments has proved to be clinically effective and less relatively toxic than the existing drugs.

In India, PUD is common, in the Indian pharmaceutical industry; anaticids and antiulcer drugs share 6.2 billion rupees and occupy 43% of the market share. Today there are two main approaches for treating peptic ulcer. The first deals with reducing the production of gastric acid and second with reinforcing gastric mucosal barrier.

A number of drugs including proton pump inhibitors and H_2 receptor antagonists are available for the treatment of peptic ulcer, but clinical evaluation of these drugs has shown incidence of relapses, side effects like arrhythmias, impotence, gynaecomastia, arthralgia, hypergastrinemia and haemopoeitic changes and drug interactions. This has been the rationale for the development of new anti ulcer drugs and we search for novel molecules has been extended to herbal drugs that offer better protection and decreased relapse.

Plan of work

The plan of work for the study of *Ficus arnottiana Miq.* was carried out as follows.

1. Collection and authentication of plant
2. Preliminary phytochemical studies
    a. Preparation of extract
    b. Qualitative phytochemical studies.

PHARMACOLOGICAL STUDIES

1) Evaluation of anti-ulcer activity of *Ficus arnottiana* Miq. by
    a. Ethanol induced ulcer model
    b. Stress induced ulcer model.
2) Determination of following parameters
    a. Ulcer index
    b. Percentage inhibition
    c. Gastric volume
    d. pH of gastric juice
    e. Total acidity
    f. Free acidity.

4. PLANT PROFILE

Ficus arnottiana miq

Scientific classification

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Order : Rosales

Family : Moraceae

Genus : Ficus

Species : ***F. arnotiana***

Binomial name **:** ***Ficus arnottiana***

Ficus arnottiana, also known as **Indian rock fig**, **rock pipal** and **Urostigma arnottiana** is a tropical fruit tree species belonging to the family Moraceae.

Small deciduous trees with milky juice and without aerial roots. Leaves alternate, broadly ovate, 7-20 x 5-15 cm, cordate at base, finely caudate at apex,

glabrous, chartaceous; margins subundulate; basal nerves 5-7, lateral nerves 8-10 pairs; reticulation fine; petioles 5-15 cm long; stipules ovate-lanceolate, 1.5 – 2.5 cm long, caducous, reddish-brown when dried. Receptacles mostly from the axils of fallen leaves, in pairs or clusters from tubercles, sessile or very shortly pedunculate, depressed globose; bracts 3. Perianth lobes 3, loose, inflated. Male flowers: few, near the mouth of the receptacles, sessile. Stamen 1. Gall and Fertile flowers: undistinguishable except by the contents of the ovary, sessile or pedicellate, the perianth gamophyllous, lax, completely investing the ovary. Ovary 1-locular; style elongate; stigma flat. Figs globose, ca 1.5 cm in diam., depressed, purple with greenish dots when ripe. Seeds many, minute.

Flowering: December – February

Fruiting : February - April.

Distribution: India: South India – Common in moist deciduous forests, open grassy slopes and in rock crevices of Western Ghats. Sri Lanka.

Uses: Leaves and twigs are lopped for fodder. Leaves and bark used in cutaneous affections.

F. arnottiana Miq is a spiny, evergreen shrub or small tree up to 15 m high, with trunk 40 cm or more in diameter; spreading crown; stipular spines and many drooping branches. The fruit is of variable shape and size. It can be oval, obovate, oblong or round, and that can be 1-2.5 in (2.5-6.25 cm) long, depending on the variety. The flesh is white and crisp. When slightly underipe, this fruit is a bit juicy and has a pleasant aroma. The fruit's skin is smooth, glossy, thin but tight.

It is most commonly found in the tropical and sub-tropical regions. Originally native to India it is now widely naturalized in tropical region from Africa to Afghanistan and China, and also through Malaysia and into Australia and in some Pacific regions. It can form dense stands and become invasive in some areas, including Fiji and Australia and has become a serious environmental weed in Northern Australia. It is a fast growing tree with a medium lifespan that can quickly reach up to 10–40 ft (3 to 12 m) tall.

Properties

It is slightly acidic in taste

Parts used

Only fruits are used. It is collected from the month of June-July.

Constituents

Ficus species contain flavanoids glycosides , alkaloids, phenolic compounds, steroids, saponins, coumarins ,tannins , triterpinoids – oleanolic acid, rusolic acid, α-hydroxy ursolic acid, protocatechuic acid, maslinicacid. The nonenzymatic constituents include phenolic compound, flavanoids, vitamin C.

Medicinal uses

Leaves used for anti-diabetic, anti-helminthic, Fruits are anti-ulcer, laxative and digestive. Bark—astringent, antiseptic, alterative, laxative, haemostatic, vaginal disinfectant (used in diabetes, diarrhoea, leucorrhoea, menorrhagia, nervous disorders; also in skin diseases.) Leaves and twigs laxative.

5. MATERIALS AND METHODS

5.1.1. PHARMACOGNOSTIC STUDIES:

a. Ash Value: (Kokate *et al*., 1985)

Principle:

The ash content of a crude drug is generally taken to be the residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also include inorganic matter added for the purpose of adulteration. There is a considerable difference varies within narrow limits in the case of the same individual drug. Hence an ash determination furnishes a basis for judging the identify and cleanliness of a drug and gives information relative to its adulteration with inorganic matter. Ash standards have been established for a number of official drugs. Usually these standards get a maximum limit on the total ash or on the acid insoluble ash permitted.

The total ash is the residue remaining after incineration. The acid insoluble ash is the part of the total ash which is insoluble in diluted hydrochloric acid.

The ash or residue yielded by an organic chemical compound is as a rule, a measure of the amount of inorganic matters present as impurity. In most cases, the inorganic matter is present is small amounts which are difficult to remove in the purification process and which are not objectionable if only traces are present. Ash values are helpful in determining the quality and purity of the crude drugs in powder form.

Procedures given in Indian pharmacopoeia were used to determine the different ash values such as total ash and acid insoluble ash.

Total Ash:

Weighed accurately about 3 gm of air dried powered drug was taken in a tarred silica crucible and incinerated by gradually increasing the temperature to make it dull red until free from carbon cooled and weighted and then calculated the percentage of total ash with reference to the air dried drug.

Acid insoluble ash:

The ash obtained as directed under total ash above was boiled with 25 ml of 2 N HCI for 5 minutes. The insoluble matter was collected on ash less filter paper, washed with hot water ignited and weighted, then calculated the percentage of acid insoluble ash with reference to the air tried drug.

Water soluble ash:

The total ash obtained was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash calculated with reference to the air dried drug.

b. Extractive values:

Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents presents in a crude drug.

Determination of alcohol soluble extractive value:

5 gm of the air-dried coarse powder of *Ficus arnottiana Miq.* was macerated with 100 ml of 90% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18 hours. Thereafter, it was filtered rapidly taking precautions against the loss of the solvent. Out of that filtrate, 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105ºC and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the air dried drug. The results are recorded in the table.

Determination of water soluble extractive value:

Weigh accurately 5 gm of coarsely powdered drug and macerate it with 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently during the first 6 hours and allow to standing for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Then 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105ºC and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drug.

c. Loss on drying:

Loss on drying is the loss in weight in percentage w/w determined by means of the procedure given below. It determines the amount of volatile matter of any kind (including water) that can be driven off under the condition specified (Desiccators or hot air oven). If the sample is in the form of large crystals, then reduce the size by quick crushing to a powder.

Procedure:

About 1.5 gm of powdered drug was weighed accurately in a tarred porcelain dish which was previously dried at 105°C in hot air oven to constant weight and then weighed. From the difference in weight, the percentage loss of drying with reference to the air dried substance was calculated.

e. Foaming index: (Divakar M.C. *et al.,* 1996)

Foaming index is mainly performed to determine the saponin content in an aqueous decoction of plant material.

Determination of foaming index:

Weighed accurately about 1g of coarsely powdered drug and transformed to 500 ml conical flask containing 100 ml of boiling water. Maintained at moderate boiling at 80-90°c for about 30 min. Cooled and added sufficient water through the filter to make up the volume to 100 ml (V_1). Cleaned 10 stoppered test tube of uniform dimension were taken and transferred the successive portions of 1, 2, 3 ml up to 10 ml and adjusted the volume of the liquid in each test tube with water to 10 ml. Stoppered the tubes and shaken them in a lengthwise motion for 15 sec uniformly and allowed to stand for 15 min and measure the height of foam. If the height of the foam in every tube is less than 1 cm, the foaming index is less than 100 (not significant). Here the foam was more than 1 cm height after dilution of plant material. If the height of the foam in every tube is more than 1 cm, the foaming index is more than 1000. In this case, 10 ml of first decoction of plant material is measured and transferred to 100 ml volumetric flask (V_2) and volume is made to 100 ml and followed the same procedure.

5.1.2. PRELIMINARY PHYTOCHEMICAL ANALYSIS

Ethanolic Extraction

About 300 gm of air dried powdered material was taken in 1000 ml soxhlet apparatus and extracted with ethanol for 2 days. The temperature was maintained at 55°C-65°C. After that extract was concentrated by distillation and solvent was recovered, the final solution was evaporated to dryness. The colour, consistency, and yield of ethanolic extract were noted.

Table 5.1: Nature and colour of extract of Ficus arnottiana Miq. lam

S. No.	Name of extract	Colour	Consistency	Yield w/w
1	Ethanolic extract	Brown	Non sticky mass	18.5

5.1.3. CHEMICAL TESTS

A) Test for Carbohydrates

1. Molisch Test

It consists of treating the compounds of α-naphthol and concentrated sulphuric acid along the sides of the test tube.

Purple colour or reddish violet colour was produced at the junction between two liquids.

2. Fehling's Test

Equal quantity of Fehling's solution A and B is added. Heat gently, brick red precipitate is obtained.

3. Barfoed's Test

To the 5 ml of the Barfoed's solution and 0.5 ml of solution under examination, heat to boiling, formation of red precipitate of copper oxide is obtained.

4. Benedict's Test

To the 5 ml of Benedict's reagent, add 8 drops of solution under examination. Mix well boiling the mixture vigorously for two minutes and then cool red precipitate is obtained

B. Test for steroids and sterols

1. Salkowski Test

Dissolve the sample of test solution in chloroform and add equal volume of concentrated sulphuric acid, cherry red, bluish red and purple colour is noted in the chloroform layer, whereas acid assumes marked green fluorescence.

2. Liebermann Burchard test

Dissolve the test sample in 2 ml of chloroform in a dry test tube. Now add 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid, the solution becomes red, then blue and finally bluish green in colour.

C) Test for alkaloids

1. Hager's Test

To the extract add 3 ml of Hager's reagent yellow precipitate is produced.

2. Mayer's Test

To the extract add 1 ml or 2 ml of Mayer's reagent. Dull white precipitate is produced.

3. Wagner's test:

To the extract add Wagner reagent, Reddish brown precipitate is produced.

4. Dragendroff's Test

To the extract, add 1 ml of Dragendroff's reagent orange red precipitate is produced.

D) Test for glycosides

1. Baljet Test

To the drug sample, sodium picrate solution is added, yellow to orange colour is produced.

2. Legal's Test

Sample is dissolved in pyridine; sodium nitropruside solution is added to it and made alkaline, pink red colour is produced.

3. Borntrager Test

Add a few ml of dilute sulphuric acid to test solution, boil, filter and extract the filtrate with ether or chloroform. Then organic layer is separated to which ammonia is added pink, red, or violet color is produced in organic layer.

4. Killer Killani Test

Sample is dissolved in acetic acid containing trace of ferric chloride and transferred to the surface of concentrated sulphuric acid. At the junction of liquid reddish brown colour is produced which gradually becomes blue.

5. Test for Saponins:

Foam Test:

About 1 ml of alcoholic sample is diluted separately with distilled water to 20 ml and shaken in graduated cylinder for 15 minutes; 1 cm layer of foam indicates the presence of saponins.

F) Test for Tri-Terpenoids

In the test tube, 2 or 3 granules of tin was added and dissolved in a 2 ml of thionyl chloride solution and test solution is added, pink colour is produced which indicates the presence of triterpenoids.

G) Test for Flavonoids

Shinoda Test: To the sample, magnesium turnings and then concentrated hydrochloric acid is added Red colour is produced.

H) Test for tannins and phenolic compounds

To 2-3 ml of extract, add few drops of following reagents,

a) 5% $fecl_3$ Solution : Deep blue – black colour

b) Lead acetate Solution : White precipitate

c) Gelatin solution : White precipitate

d) Bromine water : Decolouration of bromine water

e) Acetic acid solution : Red colour solution

f) Dilute iodine solution : Transient red colour

g) Dilute HNO3 : Reddish to yellow colour

I) Test for Fixed oils and Fatty Acids

a) Saponification Test:

Few drops of 0.5 N alcoholic potassium hydroxide are added to the extract with few drops of phenolphthalein solution, later the mixture is heated on water bath for 1-2 hour, soap formation indicates the presence of fixed oils and fats in the extract.

b) Spot test:

Small quantity of the extract is placed between two filter papers. Oil stain produced with any extract shows the presence of fixed oils and fats in the extracts.

J) Test for Gums and Mucilage

a) Ruthenium red Test

Small quantities of extract are diluted with water and added with ruthenium red solution. A pink colour production shows the presence of gums and mucilage

K) Test for proteins and Amino Acids

1) Xanthoprotein Test : To the extract, add 20% of sodium hydroxide or ammonia, orange colour indicates presence of aromatic amino acids.

2) Biuret Test: Add 1 ml of 40% of sodium hydroxide and 2 drops of 1% copper Sulphated to the extract, a violet colour indicates the presence of proteins.

3) Ninhydrin Test: Add 2 drops of freshly prepared 0.2% ninhydrin reagent to the extract and heat. A blue colour develops indicating presence of proteins, peptides or amino acids.

5.2. PHARMACOLOGICAL EVALUATION

5.2.1 Determination of LD_{50} value

Acute Oral Toxicity Study

The procedure was followed by using OECD guidelines 423 (Acute toxic class method)

The acute toxic class method is a step wise procedure with 3 animals of single sex per step. Depending on the mortality and / or moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test animals while allowing for acceptable data based scientific conclusion.

The method uses defined doses (5, 50, 300, 2000 mg / kg body weight) and the results allow a substance to be ranked and classified according to the OECD for the classification of chemical which cause acute toxicity.

Procedure:

Twelve animals (Wister Albino rats, 150-200gm) were selected for studies. The starting dose level of *Ficus arnottiana Miq.* was 50 mg/kg body weight per oral. Most of the crude extracts possess LD_{50} value more than 2000 mg /kg of the body weight of animal used.

Dose volume was administered 0.1 ml / 100 gm. body weight to the animal by oral route. After giving the dose the toxic signs were observed within 3-4 hours.

5.2.2. Evaluation of Anti-ulcer activity

Animals used

Adult Wister albino rates of either sex with weighing 150-180 gm were used. The animals were maintained on the suitable nutritional and environmental condition throughout the experiment. The animals were housed in polypropylene cages with paddy house bedding under standard laboratory condition for an acclimatization periods of 7 days prior to performing the experiment. The animal had access to laboratory chow and water, the experimental protocols were approved by institutional animal ethical committee and a written permission from in house ethical committee has been taken to carry out and complete this study.

Drugs and chemical used

Omeprazole (Omez, Reddys Laboratories, Hydrabad) was used as a standard drugs

Ethanol was used as a solvent and dried fruit of *Ficus arnottiana Miq.*

5.2.2. Experimental Procedure

A. Ethanol induced ulcer (M.A. Abdulla *et al,*) 2010)

Male albino Wister rats were divided into five groups of six animals per group and animals were fasted for 24 hours prior to the experiment in perforated steel cages to avoid coprophagy, five groups were made as below.

Group 1: received 1% CMC (1.0 ml/kg p.o.) as normal control

Group II: received 1% CMC (1.0ml/kg p.o.) as ulcer control

Group III: received (20mg/kg) per oral omeprazole as standard drug

Group IV: Received (200mg/kg) per oral ethanolic extract of *Ficus arnottiana Miq.* root.

Group V: Received (400 mg/kg) per oral ethanolic extract of *Ficus arnottiana Miq.* root.

One hour after the drug treatment the animals were treated with absolute ethanol (5ml/kg) to induce ulcers. The animals were sacrificed after 1 hour and stomach was opened and percentage inhibition of ulcer was determined.

B. Swimming Stress Induced Ulcer (Deore S L, 2009)

Stress ulcers were induced by forced swimming in the glass cylinder (height 45 cm, diameter 25 cm) containing water to the height of 35 cm maintained at 25^{o}C for 3 hours, male albino rats ware divided into five groups of six animals per group and animals were fasted for 24 hours prior to the experiment in perforated steel cages to avoid coprophagy. Five groups were made as below.

Group I: Received 1% CMC (1.0 ml/ kg p.o.) as normal control.

Group II: Received 1% CMC (1.0ml/kg p.o.) as ulcer control

Group III: Received (20 mg/kg) per oral omeprazole as standard drug.

Group IV: Received (250 mg/kg) per oral ethanolic extract or *Ficus arnottiana Miq.* fruit.

Group V: Received (500 mg/kg) per oral ethanolic extract of *Ficus arnottiana Miq.* fruit.

After the drug treatment animals were allowed to swim in water for 3 hours, then the animals were sacrificed and stomach was opened, the ulcer index and percentage inhibition of ulcer was determined.

5.2.3. BIOCHEMICAL PARAMETERS

The stomach was carefully excised keeping esophagus closed and opened along greater curvature and luminal contents were removed, the gastric contents were collected in a test tube and centrifuged. The gastric contents were analysed for gastric juice volume, pH, free and total acidity.

5.2.4. Measurement of gastric juice volume and pH

Gastric juice was collected from ethanol and stress induced ulcer rats. The gastric juice thus collected was centrifuged at 3000 rpm for 10 min. The volume of supernatant was measured and expressed as ml/100 g body weight. The pH of supernatant was measured using digital pH meter.

5.2.5. Determination of free and total acidity

An aliquot of 1.0 ml of gastric juice was pipette out into a 50 ml conical flask and 2/3 drops of topfers reagent was added to it and titrated with 0.01N NaOH until all traces of the red colour disappeared and the colour of the solution turned yellowish orange. The volume of 0.01 N NaOH was noted which corresponds to free acidity. Then 2/3 drops of phenolphthalein was added and titration was continued until a permanent pink colour was developed. The volume of total alkali consumed was noted which corresponds to total acidity. The free acidity and total acidity was determined using the formula and values are expressed as mEq/L/100g.

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100}{0.01} \text{ (mEq/L per 100g)}$$

5.2.6. Ulcer Index (UI)

The mucosa was flushed with saline and stomach was pinned on frog board, the lesion in glandular portion examined under a 10 X magnifying glass and length was measured using a divider and scale and gastric ulcers was scored, ulcer index of each animal was calculated by adding the values and their mean values were determined.

- O – Normal coloured stomach
- 0.5 – Red colouration
- 1 – Spot ulceration
- 1.5 – Hemorrhagic streak

- 2 – Ulcers
- 3 – Perforations

5.2.7. Percentage inhibition

Percentage inhibition was calculated using the following formula.

$$\% \text{ inhibition} = \frac{UI_{\text{ulcer control}} - UI_{\text{ulcer treated}}}{UI_{\text{ulcer control}}} \times 100$$

5.2.8. Statistical Analysis

All the values are expressed as mean $\pm$ S.E.M. for groups of six animals each. Analysed by one way ANOVA and compared by using Tukey - Kramer multiple comparison tests. The values are statistically significant at three levels ***P<0.001, **P<0.01, * P<0.05. But NS if P>0.05. InStat-GraphPad Prism V.5.0 software was used for statistical calculation.

6. RESULT AND DISCUSSION

The results of the present study shows that the ethanol extract of *Ficus arnottiana Miq.*fruit exerts gastro protective action against ethanol induced ulcer model and anti-secretory activity against stress induced ulcer model.

6.1. PHARMACOGNOSTICAL STUDIES:

6.1.A. ANALYTICAL PARAMETERS:

The analytical parameters were investigated and reported as, total ash value (6.76% w/w), water soluble ash value (1.98 %w/w), acid insoluble ash value (0.94%w/w), Sulphated ash value (8.81 %w/w), water soluble extractive value (9.97 %w/w), alcohol soluble extractive value (8.2 %w/w), loss on drying (5.8 %w/w). The above studies were enabled to identify the plant material for future investigation and form an important aspect of drug studies. The results were given in table.

A. ASH VALUES:

Table: Ash Values

Sl. No.	PARAMETER ASH VALUES	% w/w
1)	Total Ash	6.76
2)	Water Soluble Ash	1.98
3)	Acid Insoluble Ash	0.94
4)	Sulphated Ash	8.81

B. EXTRACTIVE VALUES:

Table: Extractive Values

Sl. No.	PARAMETER EXTRACTIVE VALUES	% w/w
1)	Water Soluble Extractive	9.97
2)	Alcohol Soluble Extractive	8.2

C. LOSS ON DRYING:

Table: Loss on drying

S.No.	PARAMETER	% w/w
1)	Loss on Drying	5.8

6.2. PRELIMINARY PHYTOCHEMICAL STUDIES

The roots of *Ficus arnottiana Miq.* were subjected for hot continuous extraction using ethanol as solvent and the yield of ethanolic extract found to be 18.5% w/w. The extracts obtained were subjected to various phytochemical tests.

Table 6.4.: Phytochemical screening of Ficus arnottiana Miq.Linn

S. No.	Phytochemical constituents	Ethanolic extract
1	Alkaloids	++
2	Saponins	--
3	Tannins	++
4	Terpenoids	--
5	Flavonoids	++
7	Glycosides	--
8	Phytosterol	++
9	Amino acids	--
10	Gums	--

6.3. PHARMACOLOGICAL STUDIES

6.3.1. Acute oral toxicity studies

The acute oral toxicity of *Ficus arnottiana Miq.*was carried out as per OECD 423 – guidelines (Acute toxic class method).

No toxicity or death was observed for these given dose levels, in selected and treated animals. Hence, the acute toxicity studies revealed that LD_{50} > 2000mg/kg for the extract.

Hence, the biological dose was fixed at two dose levels, 200 and 400 mg/kg of body weight.

6.3.2. Anti-Ulcer Screening

A. Ethanol induced ulcer

Effects of methanol extract of *Ficus arnottiana Miq.*on ulcer index induced by ethanol in rats are shown in table 6.4.

Ethanol induced gastric damage showed gross mucosal lesion, including long hemorrhage bands and petechial lesion.

Animals pretreated with methanol extract of *Ficus arnottiana Miq.*and standard drug omeprazole showed very mild lesions and somewhere no lesion at all, when compared to ulcer control group.

*Ficus arnottiana Miq.*showed a dose dependent curative ratio compared to ulcer control groups. The extracts exhibited an inhibition percentage of 62.90% and 74.62% at doses of 200 and 400 mg/kg doses respectively. The ulcer protective

action of extracts at different doses was better than that of standard drug, omeprazole, which exhibited an inhibition percentage of 83.66%

Ethanol produces severe gastric hemorrhagic lesions. The pathogenesis of ethanol induced gastric damage in rats is complicated and involves superficial aggressive cellular necrosis as well as the release of tissue derived mediators such as histamine and leukotriene C_4. These mediators act on gastric microvasculature, triggering a series of events that lead to mucosal and sub mucosal damage so the cytoprotective mechanism of *Ficus arnottiana Miq.*extract may therefore include mechanisms other than simple acid neutralization.

Table 6.5: Effect of *Ficus arnottiana Miq.*on Ulcer Index in ethanol induced gastric lesions in the rats.

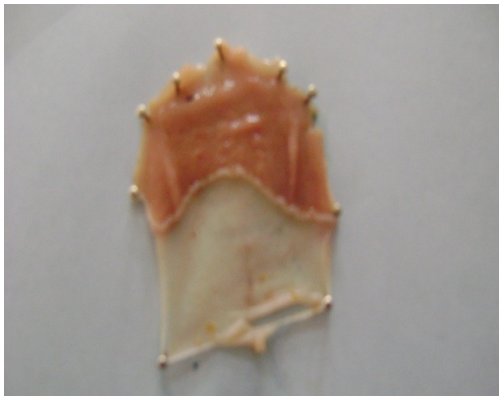
Groups	Ulcer Index	Percentage inhibition
Normal Control	00.00±0.00	--
Ulcer Control	16.47±0.63 [a]	--
Std (Omeprazole 20mglkg)	2.69±0.48 [a]	83.66%
EEFA 200mg/kg	6.11±0.41 [a]	62.90%
EEFA 400mg/kg	4.18±0.25[a]	74.62%

*Values are given as mean ± Standard error mean (S.E.M) for five groups of six animals each. Values are statistically significant at *P<0.05, **P<0.01, ***P<0.001. Ulcer control group (II) compared with normal control group (I). EEFA treated groups (IV & V) and standard group (III) were compared with Ulcer control group (II). a =p<0.001, b =p<0.01, c =p<0.05*

CONTROL ULCER CONTROL

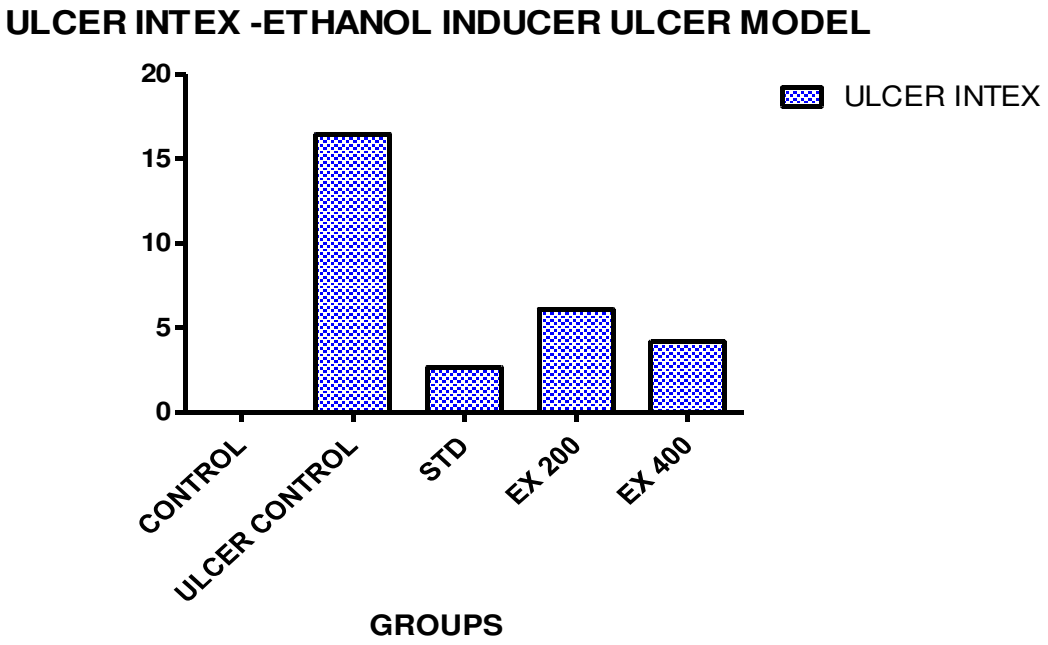
(STD OMEPRAZOLE 20 mg/Kg)

EEFA (200 mg/Kg)



EEFA (400Kg/Kg)

Fig. 6.1: Effect of *Ficus arnottiana Miq.*on ethanol induced Ulcers in rats.



Graph 6.1: Effect of *Ficus arnottiana Miq.* on Ulcer Index in Ethanol Induced Gastric Ulcer

Ulcer Index (UI) and Acid Parameters

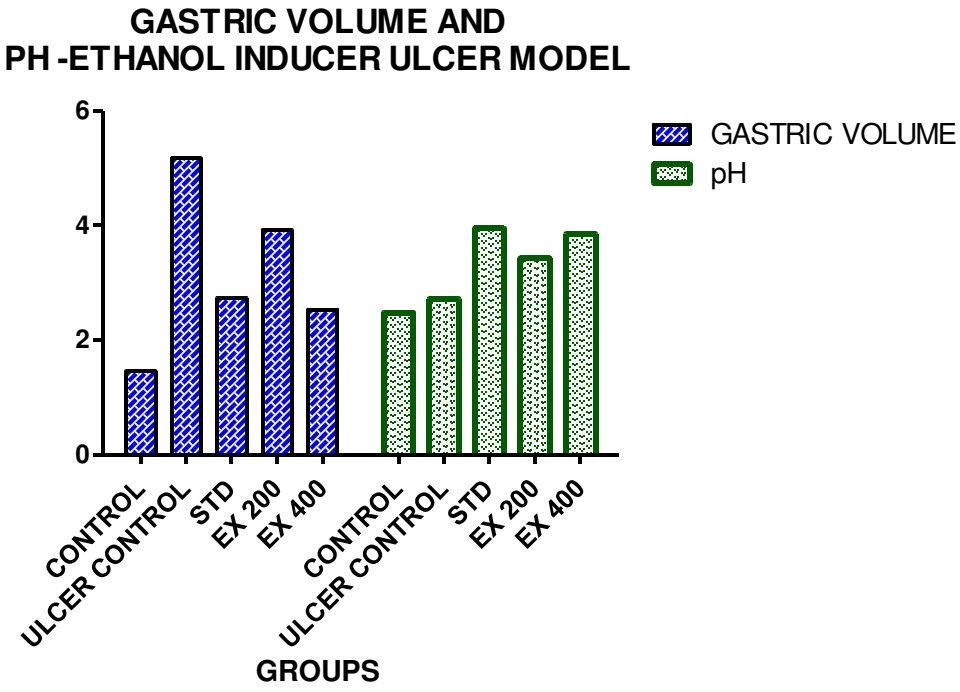
Oral administration of methanol extract of *Ficus arnottiana Miq.* at doses of 200 and 400mg/kg exhibited dose dependent inhibition percentage of 62.90% and 74.62% respectively compared to uler control, proving the antiulcer activitiy The standard drug omeprazole (20mg/kg) exhibited a percentage inhibition of 83.66%

The effect of ethnolic extract of *Ficus arnottiana Miq.* on acid parameters showed significant ($P < 0.001$) effect at 200 mg/kg and 400 mg/kg dose compared to ulcer control animals. The volume of acid secretion, total and free acidity was decreased and pH was increased when compared to ulcer control group but in this gastric environment also able to induce ulcer, so it can be thought that the antisecretory activity might not be the main mechanism of action of thease extracts.

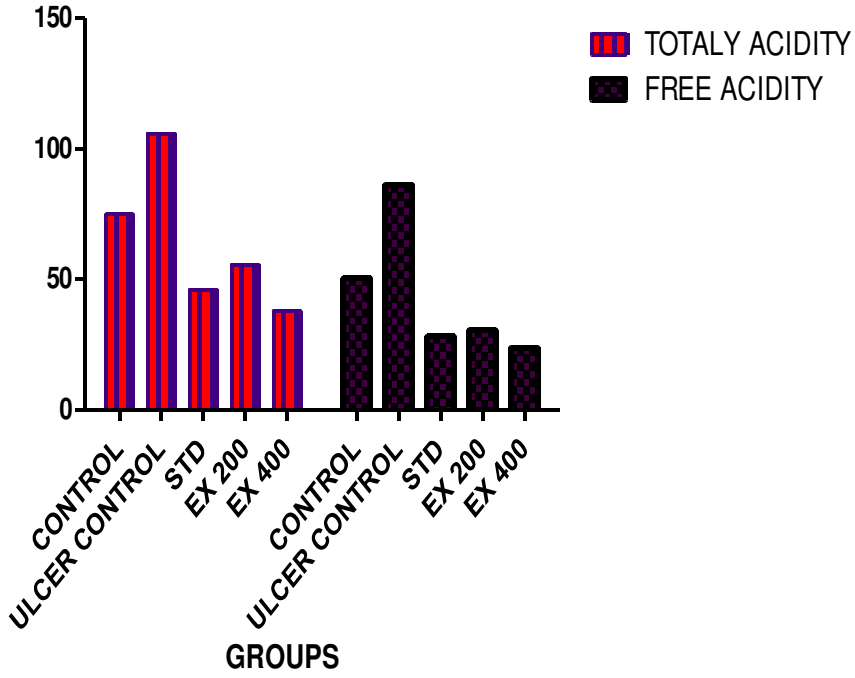
Table 6.6: Effect of *Ficus arnottiana Miq.on* volume of acid secretion, total and free acidity, and pH of the gastric juice in ethanol induced ulcers in the rats.

Groups	Gastric Volume	pH	Total Acidity	Free Acidity
Normal Control	1.46±0.11	2.48±0.02	74.97±2.57	50.39±1.72
Ulcer Control	5.18±0.46[a]	2.72±0.15 [a]	105.85±5.03 [a]	86.24±4.51 [a]
Std(Omeprazole 20mglkg)	2.74±0.15 [a]	3.95±0.30 [a]	46.07±4.04 [a]	28.14±2.08 [a]
EEFA 200mg/kg	3.92±0.25[c]	3.43±0.15 [b]	55.51±3.60 [a]	30.70±1.52 [a]
EEFA 400mg/kg	2.54±0.15 [a]	3.86±0.20 [b]	37.97±2.52 [a]	23.95±3.06 [a]

*Values are given as mean ± Standard error mean (S.E.M) for five groups of six animals each. Values are statistically significant at *P<0.05, **P<0.01, ***P<0.001. Ulcer control group (II) compared with normal control group (I). MEFA treated groups (IV & V) and standard group (III) were compared with Ulcer control group (II). a =p<0.001, b =p<0.01, c =p<0.05*


GASTRIC VOLUME AND
PH -ETHANOL INDUCER ULCER MODEL
6
4
2
0
GASTRIC VOLUME
pH
CONTROL
ULCER CONTROL
STD
EX 200
EX 400
CONTROL
ULCER CONTROL
STD
EX 200
EX 400
GROUPS

TOTAL ACIDITY & FREE ACIDITY ETHANOL INDUCED ULCER MODEL



B. Swimming Stress Induced Ulcer

Oral administration of ethonolic extract of *Ficus arnottiana Miq.* 1h before the induction of stress, reduced the water immersion stress induced ulcers. The ethanolic extract of *Ficus arnottiana Miq.*exhibited a dose dependent inhibitin percentage of 46.92% and 72.91% at doses of 200 and 400 mg/kg dose respectively the standarad drug omeprazole showed an inhibition percentage of 78.13%. The results are shown in table.6.7

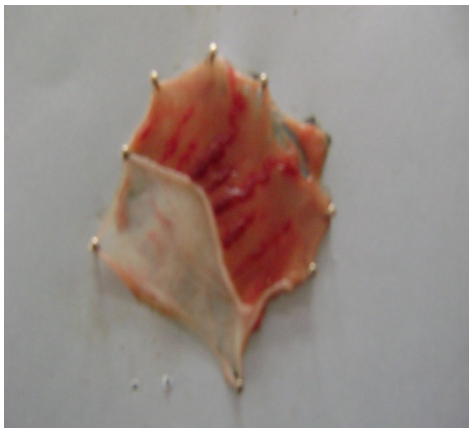
Water immersion stress induced ulcer is one of the best models to induce ulcer in rats. The model provides both emotional stress as well as physiological stress to the animal. Omeprazole was used here to study the proton pump inhibitor mechanism water immersion stresss induced ulcers are result of autodigestion of gastric mucosal barrier and accumulation of HCL in the stomach. The ethanol extract of *Ficus arnottiana Miq.* prevents autodigestion of gastric mucosal barrier in a dose dependend manner in swimming stress induced ulcers due to its cytoprotective action.

Table 6.7 Effect of *Ficus arnottiana Miq.*on Ulcer Index in stress induced gastric lesions in the rats

Groups	Ulcer Index	Percentage inhibition
Normal Control	00.00±0.00	--
Ulcer Control	16.28±2.08	--
Std(Omeprazole 20mglkg)	3.56±0.96[a]	78.13%
EEFA 200mg/kg	8.64±1.25 [b]	46.92%
EEFA 400mg/kg	4.41±1.00[a]	72.91%

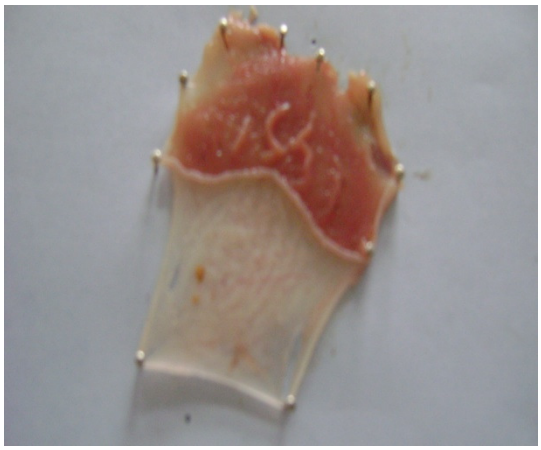
*Values are given as mean ± Standard error mean (S.E.M) for five groups of six animals each. Values are statistically significant at *P<0.05, **P<0.01,aP<0.001. Ulcer control group (II) compared with normal control group (I). MEFA treated groups (IV & V) and standard group (III) were compared with Ulcer control group (II). a =p<0.001, b =p<0.01, c =p<0.05*

CONTROL

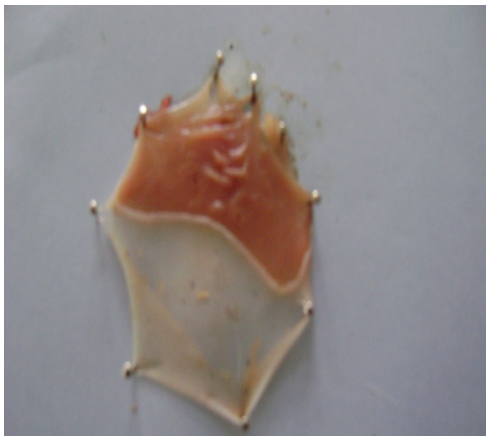


ULCER CONTROL

STD (OMEPRAZOL2-mg/Kg)

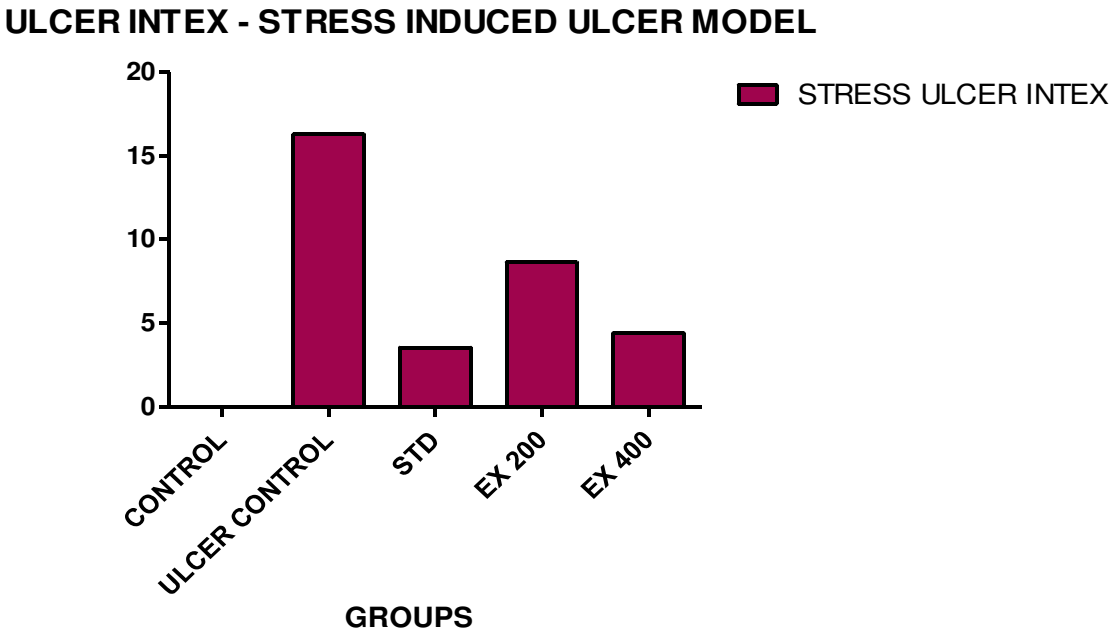


EEFA (200 mg/Kg)



EEFA (400 mg/Kg)

Figure : 6.2. Effect of *Ficus arnottiana Miq.*on stress induced Ulcers in rats.



Graph 6.2: Effect of *Ficus arnottiana Miq.*on ulcer index in

Swimming stress induced gastric ulcer

Ulcer Index (UI) and Acid Parameters

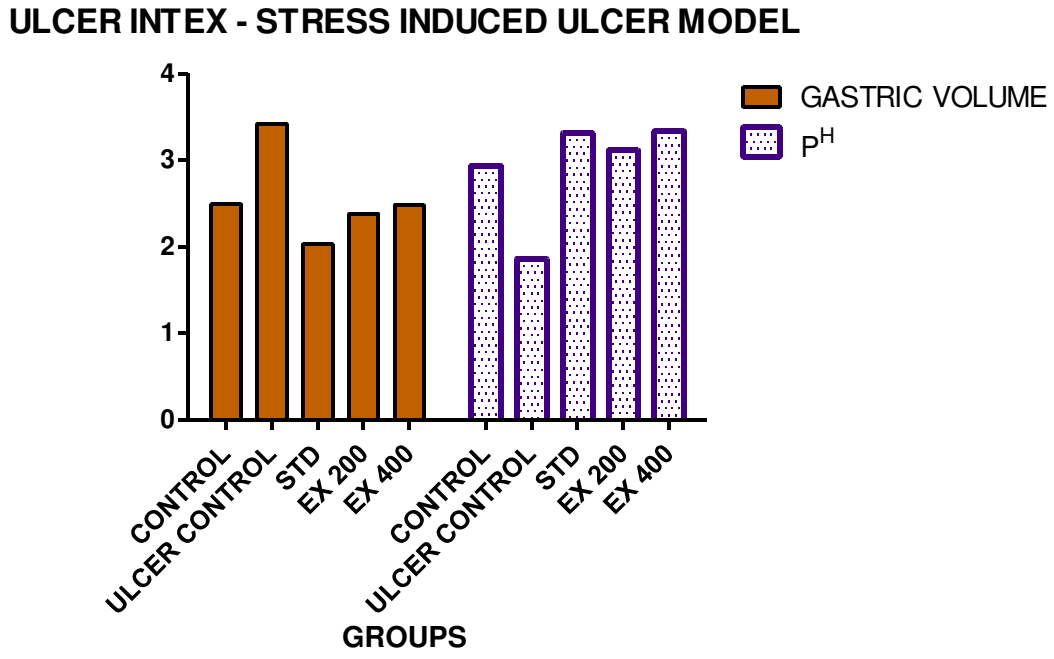
Oral administration of methanol extract of *Ficus arnottiana Miq.*at doses of 200 and 400 mg/kg exhibited dose dependent inhibition percentage of 46.92% and 72.91% respectively compared to ulcer control proving the antiulcer activity. The standard drug omeprazole (20mg/kg) exhibited a percentage inhibition of 78.13%

The effects of ethanolic extract of *Ficus arnottiana Miq.*on acid parameters showed significant ($P<0.01$) and ($P < 0.001$) effect at 200mg/kg and 400mg/kg dose respectively compared to ulcer control animals. The volume of acid secretion, total and free acidity was decreased and pH of the gastric juice was increased compared to ulcer control group. But in this gastric environment also able to induce ulcer, so it can be thought that the anti-secretory activity might not be the main mechanism of action of these extracts.

Table 6.8: Effect of *Ficus arnottiana Miq.*on volume of acid secretion, total and free acidity, and pH of the gastric juice in stress induced ulcers in the rats.

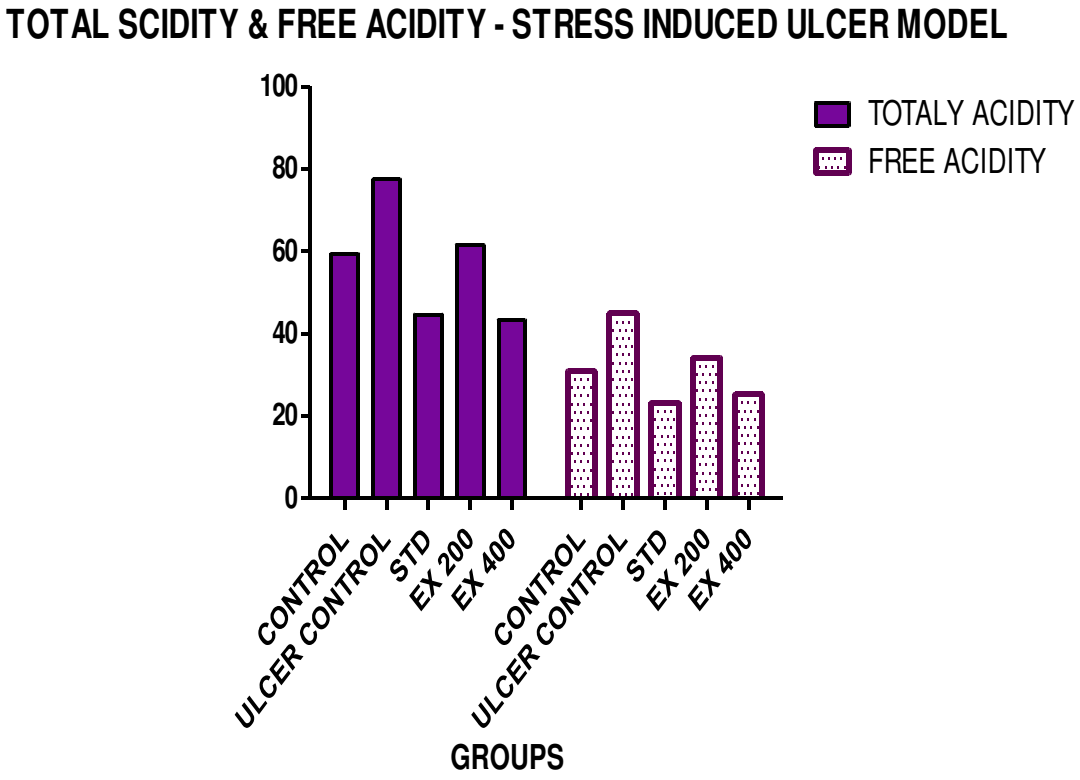
Groups	Gastric Volume	pH	Total Acidity	Free Acidity
Normal Control	2.49±0.04	2.94±0.34	59.36±2.00	30.95±0.96
Ulcer Control	3.42±0.29[a]	1.86±0.09[a]	77.46±3.47[b]	44.93±2.22[a]
Std(Omeprazole 20mglkg)	2.03±0.07[a]	3.32±0.16[a]	44.61±3.512[a]	23.20±2.08[a]
MEZM 200mg/kg	2.38±0.06[a]	3.12±0.20[b]	61.58±3.61[b]	34.22±1.16[b]
MEZM 400mg/kg	2.48±0.07[a]	3.34±0.19[a]	43.26±2.08[a]	25.29±2.64[a]

*Values are given as mean ± Standard error mean (S.E.M) for five groups of six animals each. Values are statistically significant at *P<0.05, **P<0.01,aP<0.001. Ulcer control group (II) compared with normal control group (I). MEZM treated groups (IV & V) and standard group (III) were compared with Ulcer control group (II). a =p<0.001, b =p<0.01, c =p<0.05*



Graph 6.3: Effect of *Ficus arnottiana Miq.*on ulcer index in

Swimming stress induced gastric ulcer



Graph 6.6: Effect of *Ficus arnottiana Miq.*on ulcer index in Swimming stress induced gastric ulcer

7. SUMMARY AND CONCLUSION

The present study was undertaken to determine the antiulcer activity of ethanol extract from the roots of *Ficus arnottiana*.

The preliminary phytochemical investigation showed the presence of alkaloids, saponins, flavonoids, carbohydrates, cardiac glycosides, tannins and phytosteroids.

The pharmacological studies of ethanol extract were performed by the following OECD-423 guidelines.

The phytoconstituents like flavonoids, tannins and saponins have been reported in several anti-ulcer literatures as possible gastro protective agents, flavonoids, tannins and saponins are among the cytoprotective active materials for which antiulcergenic efficacy has been confirmed.

It is suggested that these compounds will be able to stimulate mucus, bicarbonate and prostaglandin secretion and counteract with the deteriorating effects of relative oxidants in gastrointestinal lumen. Tannins may prevent ulcer development due to their protein precipitating and vasoconstriction effects. Their astringent action can help precipitating micro proteins on the ulcer site, thereby forming an impervious layer over the lining that hinders gut secretions and protects the underlying mucosa from toxins and other irritants. Alkaloids prevent ulcer induced by stress.

The acute toxicity studies for this extract showed that there was no toxicity signs were produced. (LD_{50} value is 2000 mg/kg.)

Similarly the methanol extract of *Ficus arnottiana* fruit showed the presence of flavonoids and their glycosides, tannins, alkaloids and saponins. These phytoconstituents present in the extract could be possible agents involved in the prevention of gastric lesions induced by ethanol and stress induced ulcer model.

Ficus arnottiana showed a dose dependent curative ratio compared to ulcer control groups. The extracts exhibited an inhibition percentage of 62.90% and 74.62 at doses of 200mg/kg and 400mg/kg doses respectively. The ulcer protective action of extracts at 400mg/kg was comparable to that of standard drug omeprazole, which exhibited an inhibition percentage of 83.66. Oral administration of ethanolic extract of *Ficus arnottiana* 1h before the induction of stress reduced the water immersion stress induced ulcers. The ethanolic extract of *Ficus arnottiana* exhibited a dose dependent inhibition percentage of 46.92 % and 72.91% at doses of 200 & 400mg/kg dose respectively. This may be due to proton pump inhibitory activity of the extract. The standard drug omeprazole showed an inhibition percentage of 78.13%.

Further pharmacological and biochemical investigation are to be done to find out the active constituent responsible for the anti ulcer activity and elucidate the possible mechanism of action.

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